(D3)

EBI
(# 193)

85,5 % ≡ SEQ 1.
over 484 ntd

ID AAZ32041 standard; DNA; 495 BP.
XX
AC AAZ32041;
XX
DT 10-JAN-2000 (first entry)
XX
DE Human METH2 related clone HCESF90R.
XX
KW Human; METH1; METH2; anti-angiogenic; metalloprotease thrombospondin;
KW cancer; diagnosis; hyperproliferative disorder; autoimmune disease;
KW angiogenesis inhibitor; abnormal wound healing; inflammation;
KW rheumatoid arthritis; psoriasis; endometrial bleeding disorder;
KW diabetic retinopathy; macula degeneration; haemangioma; detection;
KW arterial-venous malformation; immune deficiency; ss.
XX
OS Homo sapiens.
XX
PN WO9937660-A1.

XP-002356217

XX
PD 29-JUL-1999.
XX
PF 22-JAN-1999; 99WO-US001313.
XX
PR 23-JAN-1998; 98US-0072298P.
PR 28-AUG-1998; 98US-0098539P.
XX
PA (IRUE/) IRUELA-ARISPE L.
PA (HAST/) HASTINGS G A.
PA (RUBE/) RUBEN S M.
XX
PI Iruela-Arispe L, Hastings GA, Ruben SM;
XX
DR WPI; 1999-590684/50.
XX
PT New isolated metalloprotease thrombospondin polypeptides, useful for
PT treating hyperproliferative disorders, cancers or autoimmune disorders.
XX
PS Disclosure; Page 404; 457pp; English.
XX
CC AAZ32000 and AAZ32001 encode, and AAY49501 and AAY49502 represent, human
CC metalloprotease thrombospondin (METH) proteins METH1 and METH2
CC respectively. METH1 and METH2 have been found to be potent inhibitors of
CC angiogenesis both in vitro and in vivo. They can be used for treating
CC cancer and other disorders related to angiogenesis including abnormal
CC wound healing, inflammation, rheumatoid arthritis, psoriasis, endometrial
CC bleeding disorders, diabetic retinopathy, some forms of macula
CC degeneration, haemangiomas, and arterial-venous malformations. They may
CC be useful in treating deficiencies or disorders of the immune system, by
CC activating or inhibiting the proliferation, differentiation, or
CC mobilisation (chemotaxis) of immune cells. The etiology of these immune
CC deficiencies or disorders may be genetic, somatic, such as cancer or some
CC autoimmune disorders, acquired (e.g. by chemotherapy or toxins), or
CC infectious. They can also be used to treat inflammatory conditions, both
CC chronic and acute conditions. The products can also be used for detection
CC and diagnosis. AAZ32002 to AAZ32080, and AAY49503 to AAY49511 represent
CC sequences given in the exemplification of the present invention
XX
SQ Sequence 495 BP; 95 A; 135 C; 117 G; 127 T; 0 U; 21 Other;

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aattcggcac gagcaaagtt ctgcgctcca ttgtgggcat caaacgacac gtcaaagccc        60
tccatctggg ggacacagtg gactctgatc agttcaagcg ggaggaggat ttctactaca       120
cagaggtgca gctgaaggag gaatctgctg ctgctgctgc tgctgctgcc gcagacnccc       180
agtccctggg actcccacct ccgagccagc tcccaccccc agcatgactg gcctgcctct       240
gtctgctctt ccaccacctc ttgcacaaag cccagtcctc cggcccagaa catcctgggc       300
ccggagttcc ttccttgcct tnaggggntt ttcagcaagt tnagttcctt gggtcctttt       360
tgggaaantt naggnagttn aaggantacc aggttnttgc catnctttcc agatccaagt       420
ttnacnaaaa attttnaaca gtntaaattg ggtttnttgn ccctttnngg nggntgtttt       480
ttttttcggg tccgg                                                       495
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PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

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(57) Abstract

The present invention relates to novel anti-angiogenic proteins, related to thrombospondin. More specifically, isolated nucleic acid molecules are provided encoding human METH1 and METH2. METH1 and METH2 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. Also provided are diagnostic methods for the prognosis of cancer and therapeutic methods for treating individuals in need of an increased amount of METH1 or METH2.

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Language: English

Attorney/Agent: STEFFE, Eric, K.; Sterne, Kessler, Goldstein & Fox P.L.L.C., Sui (US).

ABSTRACT:

The present invention relates to novel anti-angiogenic proteins, related to thrombospondin. More specifically, isolated nucleic acid molecules are provided encoding human METH1 and METH2. METH1 and METH2 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. Also provided are diagnostic methods for the prognosis of cancer and therapeutic methods for treating individuals in need of an increased amount of METH1 or METH2.

La présente invention concerne de nouvelles protéines anti-angiogéniques liées à la thrombospondine. Plus spécifiquement, cette invention concerne des molécules d'acide nucléique isolées codant les METH1 et les METH 2 humaines. De même, l'invention concerne des polypeptides METH1 et METH2, des vecteurs, des cellules hôtes ainsi que des procédés de recombinaison permettant de les produire. Enfin, l'invention concerne des méthodes diagnostiques utilisées pour établir les pronostics du cancer et des méthodes thérapeutiques utilisées pour traiter des sujets ayant besoin d'une grande quantité de METH1 ou METH2.

METH1 and METH2 Polynucleotides and Polypeptides Background of the Invention Federally-Sponsored Research and Development Part of the work performed during development of this invention utilized U. S. Government funds.

The U. S. Government has certain rights in this invention.

Field of the Invention The present invention relates to novel anti-angiogenic proteins, related to thrombospondin. More specifically, isolated nucleic acid molecules are provided encoding human METHI and METH2 (ME, for metalloprotease, and TH, for thrombospondin). METHI and METH2 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. Also provided are diagnostic methods for the prognosis of cancer and therapeutic methods for treating individuals in need of an increased amount of METHI or METH2.

RelatedArt Angiogenesis, the formation of new blood vessels from pre-existing vasculature, is a tightly regulated process in normal adults. Under physiological circumstances, growth of new capillaries is tightly controlled by an interplay of growth regulatory proteins which act either to stimulate or to inhibit blood vessel growth. Normally, the balance between these forces is tipped in favor of inhibition and consequently blood vessel growth is restrained. Under certain pathological circumstances, however, local inhibitory controls are unable to restrain the increased activity of angiogenic inducers. Angiogenesis is a key step in the metastasis of cancer (Folkman, Nature Med 1:27-31 (1995)) and in abnormal wound healing, inflammation, rheumatoid arthritis, psoriasis, and diabetic retinopathy, it is integral to the pathology (Folknian el a/., Science 235:442-447 (I 987)), engendering the hope that these pathological entities could be regulated by pharmacological and/or genetic suppression of blood vessel growth (1ruela- Arispe el aL, Thromb. Haem. 78:672-677 1997)).

Thrombospondin-I (TSP-1) is a 450 kDa, anti-anglogenic adhesive glycoprotein released from activated platelets and secreted by growing cells (reviewed in Adams, Int. J Biochem. Cell. Biol. 29:861-865 (1997)). TSP-1 is a homotrimer, with each subunit comprised of a 1152 amino acid residue polypeptide, post-translationally modified by N-linked glycosylation and beta- hydroxylation of asparagine residues.

TSP-1 protein and mRNA levels are regulated by a variety of factors.

TSP-1 protein levels are'downregulated by IL-1 alpha and TNF alpha. TSP-1 mRNA and protein levels are upregulated by polypeptide growth factors including PDGF, TGF-beta, and bFGF (Bornstein, Faseb J 6: 3290-3299 (1992)) and are also regulated by the level of expression ofthe p53 tumor suppressor gene product (Dameron el aL, Science 265:1582-1584 (1994)). At least four other members of the thrombospondin family have been identified: TSP-2, TSP-3, TSP-4, and TSP-5 (also called COMP). There is a need in the art to identify other molecules involved in the regulation of angiogenesis.

Summary of the Invention The present invention provides isolated nucleic acid molecules comprising a polynucleotide encoding the MIETHI polypeptide having the an-tino acid sequence shown in SEQ ID NO:2 or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit Number 209581 on January 15, 1998.

The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding the METH2 polypeptide having the amino acid sequence shown in SEQ ID NO: 4 or the amino acid sequence encoded by the cDNA clone deposited in a bacterial host as ATCC Deposit Number 209582 on January 15, 1998.

The present invention also relates to recombinant vectors, which include the isolated nucleic acid molecules of the present invention, and to host cells containing the recombinant vectors, as well as to methods of making such vectors and host cells and for using them for production of NMTHI or METH2 polypeptides or peptides by recombinant techniques.

The invention further provides an isolated NMTH I or NffiTH2 polypeptide having an amino acid sequence encoded by a polynucleotide described herein.

The invention further provides a diagnostic method useful during diagnosis or prognosis of cancer.

t 0 An additional aspect of the invention is related to a method for treating an individual in need of an increased level of METH I or NffiTH2 activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an isolated WTHI or NMTH2 polypeptide of the invention or an agonist thereof Brief Description of the Figures Figure I shows the nucleotide (SEQ ID NO: 1) and deduced amino acid (SEQ ID

NO:2) sequences of METHL The protein has a predicted leader sequence of about 28 amino acid residues (underlined).

Figure 2 shows the nucleotide (SEQ ID NO:3) and deduced amino acid (SEQ ID NOA) sequences of METH2. The protein has a predicted leader sequence of about 23 amino acid residues (underlined).

Figure 3 shows a comparison ofthe amino acid sequence ofMETH I (SEQ ID NO:2) and METH2 (SEQ ID NOA) with that of their closest homologue, a bovine metalloprotease (pNPI) (SEQ ID NO:5). Identical amino acids are boxed.

Functional domains predicted by sequence and structural homology are labeled, including the signal peptide (single line), the potential cleavage site for mammalian subtilisin (double underlined), the zinc-binding-site (dotted line) in the metalloprotease domain, and the putative disintegrin loops (arrows).

Figure 4 shows the primary structure of NIETHI, NIETH2 and pNPI which includes a prodomain, a catalytic metalloprotease domain, a cysteine rich disintegrin domain, a TSP-like domain, a spacer region and a different number of TSP-like domains, three for NIETHI, two for METH2, and four for pNPI.

Figure 5 shows a comparison of the TSP-like domain of METH I (SEQ ID NO:2) and METH2 (SEQ ID NOA) with those of TSP I (SEQ ID NOs: 6, 7, and 8) and TSP2 (SEQ ID NOs:9, 10, and 11), cysteines are numbered I to 6, tryptophans are marked by asterisks.

Figure 6 shows that peptides and recombinant protein derived from the TSP-like domain of METHI and METH2 block VEGF-induced anglogenesis.

Angiogenesis was induced on CAMs from 12 day-old embryos using a nylon mesh containing VEGF casted on matrigel and in the presence or absence of the peptides or recombinant protein. Capillary density was evaluated as described in Example 4. Positive and negative control included VEGF alone and vehicle alone, respectively. (A) Quantification of the angiogenic response induced by VEGF in the presence of recombinant proteins. TSPI, purified platelet TSPI, GST, purified GST, GST-TSPI, GST-NIETHI, and GST-METH2 are described in Example 4. (B) Quantification of the anglogenic response induced by VEGF in the presence or absence of the peptides; P-TSPI, P-NMTHI, and P-NEETH2 (peptide derived from the Type I repeats of TSP, NIETHI and NIETH2, respectively); SCI and SC2 are scramble peptides used as controls. (C) Dose@ response of the VEGF-induced angiogenesis in the presence of GST-MIETHL (D) Dose-response of the VEGF-induced angiogenesis in the presence of GST- METH2. The angiogenic index was expressed considering the vascular response from the VEGF-matrigel as I 00% and subtracting the background levels (matrigel alone). Assays were repeated, at least, twice. Each treatment was done in triplicate. Values represent the mean, bars indicate standard deviations. *p<O. 00 1.

Figure 7 shows the effect of NMTH I and METH2 recombinant proteins on bFGF-stimulated cell proliferation. Cells were cultured on 24-well plates in media containing bFGF and the recombinant protein to be tested (3 @tg/ml, unless indicated in the graph). Controls included vehicle or GST recombinant protein alone. (A), HDEC, human dermal endothelial cells; (B), FEMEC, human mammary epithelial cells; (C), HDF, human dermal fibroblasts- (D), SMC, smooth muscle cells; (E) Dose-response of GST-METHI and GST-NMTH2 on HDEC proliferation. Experiments were repeated, at least, twice. Each treatment was done in triplicate. Values represent the mean, bars indicate standard deviations.

*p<0 Figure 8 shows a schematic representation of the pHE4-5 expression vector (SEQ ID NO: 12) and the subcloned MIETH I or METH2 cDNA coding sequence. The locations of the kanamycin resistance marker gene, the METHI or METH2 coding sequence, the oriC sequence, and the laclq coding sequence are indicated.

Figure 9 shows the nucleotide sequence of the regulatory elements of the pHE promoter (SEQ ID NO: 13). The two lac operator sequences, the Shine-Delgarno sequence (S/D), and the terminal HindIII and NM restriction sites (italicized) are indicated.

Figure IO shows an analysis of the METH I amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity- amphipathic regions; flexible regions; antigenic index and surface probability are shown, and all

were generated using the default settings. In the "Antigenic Index or Jameson-Wolf'graph, the positive peaks indicate locations of the highly antigenic regions of the METHI or METH2 protein, i.e., regions from which epitope-bearing peptides of the invention can be obtained. The domains defined by these graphs are contemplated by the present invention. Tabular representation of the data summarized graphically in Figure IO can be found in Table 1.

Figure I I shows an analysis of the NMTH2 amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, and all were generated using the default settings. In the "Antigenic Index or Jameson-Wolf 'graph, the positive peaks indicate locations of the highly antigenic regions of the METHI or METH2 protein, i.e., regions from which epitope-bearing peptides of the invention can be obtained. The domains defined by these graphs are contemplated by the present invention. Tabular representation of the data summarized graphically in Figure I I can be found in Table 2.

Table 1 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Met I A A 0.41 Gly 2 A C 0.91 Asn 3 A A 0.71 Ala 4 A A 0.89 Glu 5 A A 0.93 Arg 6 A B 1.23 Ala 7 B T 1.69 I 0 Pro 8 T T 1.39 Gly 9 T T 1.28 Ser 10 T T 0.93 Arg I I T T 0.61 Ser 12 T T 0.34 5 Phe 13 B T 0.34 Gly 14 B T 0.38 Pro 15 B B 13 Val 16 B B 06 Pro 17 B B -1,57 Thr 18 A B 68 Leu 19 A B 92 Leu 20 A A 30 Leu 21 A A 03 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Leu 22 A A 63 Ala 23 A A - 3.13 Ala 24 A A 91 Ala 25 A A - 2.96 Leu 26 A A B 44 Leu 27 A A B 63 Ala 28 A A B 63 Val 29 A A B 86 17i Ser 30 A A 61 @i I tIi 10 Asp 31 A A 69 Ala 32 A A 09 Leu 33 A C 0.20 Gly 34 A c 1.06 Arg 35 T c 1.36 1 5 Pro 36 T c 1.36 Ser 37 T c 1.94 Glu 38 A T 2.76 Glu 39 A A 2,29 Asp 40 A A 1.32 Glu 41 A A 0.68 Glu 42 A A 0.77 Leu 43 A A 0.77 I Res Pos. Garni.. Chou-... Garni.. Chou,. Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Val 44 A A - 0.04 Val 45 A A 04 Pro 46 A A 0.07 Glu 47 A 52 Leu 48 A 0.08 Glu 49 A 0.59 Arg 50 A 1.41 Ala 51 A T 1.28 Pro 52 T T 0.97 I 0 Gly 53 T T 1.47 His 54 T C 1.58 Gly 55 C 0.66 Thr 56 C 1.36 Thr 57 A B 0.76 5 Arg 58 A B 1.07 Leu 59 A B 0.51 Arg 60 A B 0.16 Leu 61 A B 0.47 His 62 A B 0.78 Ala 63 A A 0.67 64 A A 65 A _r A Res Pos. Garni.. Chou-... Garni.. Chou,. Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gin 66 A A 0.56 Gin 67 A A 0.59 Leu 68 A A 0.37 Asp 69 A A 1.18 Leu 70 A B 0.97 Glu 71 A B 0.97 Leu 72 A B 0.67 Arg 73 T c 1.18 Pro 74 T T 048 Asp 75 T T 0.48 Ser 76 T c 11 Ser 77 B 0.49 Phe 78 B 0.03 Leu 79 B 46 Ala 80 B T 77 Pro 81 B T 28 Gly 82 T T 98 Phe 83 B T 28 Thr 84 B B 32 Leu 85 B B 08 Gin 86 B B 0.24 87 B T 0.63 Asn I I 1 - I I Res Pos. Garni.. Chou-... Garni.. Chou,. Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Val 88 B T 1.03 Gly 89 T c 1.00 Arg 90 T T 1.51 Lys 91 T C 1.51 Ser 92 T c 1,20 Gly 93 T c 1.84 Ser 94 T C 1.38 Glu 95 c 1.06 17@ Thr 96 c 1,01 I 0 Pro 97 C 1.00 Leu 98 c 1.34 Pro 99 A 0.83 Glu 100 A A 0.24 Thr 101 A A 0.52 Asp 102 A A 0.07 Leu 103 A A 0.18 Ala 104 A A 0.14 His 105 A B 16 cys 106 A B 19 Phe 107 A B 50 Tyr 108 B T 54 Ser 1,09 T T T 0.04 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gly 110 T T 27 Thr III T T 0.40 Val 112 B B 0.89 Asn 113 B T 0.83 Gly 114 B c 0.83 Asp 115 T c 0.59 Pro 116 T C 0.31 Ser 117 T c 0.58 Ser 118 A T 23 A Ala 119 A 19 Ala 120 A A 00 Ala 121 A A 46 Leu 122 A A 16 Ser 123 A A 20 Leu 124 A A 47 cys 125 A B 77 Glu 126 A B 52 Gly 127 A 30 Val 128 A 70 Arg 129 B 13 Gly 130 B B 28 Ala 131 B B 09 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Phe 132 B B 09 Tyr 133 B B 23 Leu 134 A B B 93 Leu 135 A B B 83 Gly 136 A A B 94 Glu 137 A A 13 Ala 138 A A B 89 Tyr 139 B B 29 Phe 140 B B 29 I 0 Ile 141 B B 16 Gin 142 B B 74 Pro 143 B B 74 Leu 144 A c 80 Pro 145 A c 10 Ala 146 A A 0.90 Ala 147 A A 0.09 Ser 148 A A 29 Glu 149 A A 0.21 Arg 150 A A 17 Leu 151 A A 17 Ala 152 A A 0.21 Thr 1 153 A A 0.17 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ala 154 A A 0.17 Ala 155 T C 0.10 Pro 156 T C 0.70 Gly 157 T T 1.08 Glu 158 T C 0.80 Lys 159 c 1.18 Pro 160 C 0.96 C 1.17 Pro 161 114 Ala 162 A A 0.81 Pro 163 A A 0.78 08 Leu 164 A A Gin 165 A A 68 36 Phe 166 A B His 167 A B 0.34 Leu 168 A B 0.56 Leu 169 A B 1.48 Arg 170 T T 1.48 Arg 171 T T 1.83 Asn 172 T T 1.87 Arg 173 T T 1.82 Gin 174 T 2.29 Gly 175 T 1.83 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asp 176 T T 1.41 Val 177 B T 0.74 Gly 178 T T 0.29 Gly 179 B T 57 Thr 180 B B 08 cys 181 B B 08 Gly 182 B B 22 114 Val 183 B B 0.12 01.4 Val 184 B B 0.26 I 0 Asp 185 B T 0.68 Asp 186 B T 1.13 Glu 187 B T 1.17 Pro 188 T c 1.68 Arg 189 T c 2.58 Pro 190 T c 1.99 Thr 191 T c 1.99 Gly 192 T c 1.68 Lys 193 A A 1.89 Ala 194 A A 1.78 Glu 195 A A 1.99 Thr 196 A A 2.30 Glu 197 A A 2.64 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asp 198 A A 2.26 Glu 199 A A 2.53 Asp 200 A T 2.53 Glu 201 A T 2.50 Gly 202 A T 2.50 Thr 1 203 A T 2.50 Glu 204 A A 2.50 Gly 205 A A 2.16 Glu 206 A A 1.94 I 0 Asp 207 A T 2.29 Glu 208 A c 2.31 Gly 209 T C 2.01 Pro 210 T T 2.14 Gln 211 T T 2.14 5 Trp 212 T C 2.14 Ser 213 C 1.93 Pro 214 T T 1.69 Gln 215 T C 1.09 Asp 216 T C 1.09 Pro 217 T c 1.03 Ala 218 r 0.48 Leu 219 B 0.34 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gin 220 B 0.34 Gly 221 B T 0.13 Val 222 B T 0.03 Gly 223 B T 0.28 Gin 224 B T 0.78 Pro 225 B 0.43 Thr 226 T 0.48 Gly 227 T c 0.44 (7i Thr 228 B T 0.90 I 0 Gly 229 B T 0.94 Ser 230 B T 1.20 lie 231 A B 1.62 Arg 232 A B 1.27 Lys 233 A B 0.72 5 Lys 234 A B B 0.77 Arg 235 A B B 0.77 Phe 236 B B 1.62 Val 237 B B 1.62 Ser 238 B T 1.33 Ser 239 T c 0.43 His 240 T c 0.32 Arg 1 241 T c 0.71 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Tyr 242 A B 0.97 Val 243 A B 0.46 Glu 244 B B 10 Thr 245 B B 66 Met 246 A B B 77 Ln Leu 247 A B 52 Val 248 A B 0.03 Ala 249 A B 57 Asp 250 A T 84 I 0 Gln 251 A T 24 Ser 252 A T 13 Met 253 A T 0.69 Ala 254 A 0.93 Glu 255 A 0.63 5 Phe 256 A 0.29 His 257 A T 22 Gly 258 A T 0.42 Ser 259 1 A T 0.98 Gly 260 A T 0.73 Leu 261 A A 0.62 Lys 262 A A 16 His 263 A B Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Tyr 264 A B 63 Leu 265 A B 99 Leu 266 A B 48 Thr 267 A B 38 Leu 268 A B 93 Phe 269 A A 28 Ser 270 A A 36 Val 271 A A 36 Ala 272 A A 29 I 0 Ala 273 A A 43 Arg 274 A A 0.23 Leu 275 A A 0.32 Tyr 276 T 0.88 0.58 Lys 277 B 5 His 278 B T 1.28 Pro 279 B T 1.17 Ser 280 T T 1.68 Ile 281 B T 1.07 Arg 282 B B 0.72 Asn 283 B B 06 Ser 284 B B 70 Val 285 B B 26 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ser 286 B B 22 Leu 287 B B 29 Val 288 B B 18 Val 289 B B 69 Val 290 B B 69 Lys 291 B B 28 Ile 292 B B 50 Leu 293 B B 64 Val 294 B B 79 Ile 295 B B 0.07 His 296 A B 0.07 Asp 297 A B 0.61 1.21 Glu 298 A GIn 299 T 2.07 5 Lys 300 C 2.10 Gly 301 T C 1.82 Pro 302 T C 1.52 Glu 303 B T 1.52 Val 304 A T 0.93 Thr 305 A T 0.30 Ser 306 A T 17 T 27 rAsn 307 A Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ala 308 A T 08 Ala 309 A 11 Leu 310 A 0.20 Thr 311 B 20 Leu 3 12 B -0,87 Arg 313 B 28 Asn 314 T 0.02 Pile 315 T T 0.83 cys 316 T T 1.19 I 0 Asn 317 T T 2.00 Trp 318 T T 1.86 Gin 319 T 1.86 Lys 320 T 2.34 Gin 321 T 2.90 5 His 322 C 2.50 Asn 323 c 2.79 Pro 324 T c 2.90 Pro 325 T T 2.86 Ser 326 T C 2.27 Asp 327 T c 2.30 Arg 328 A A 2.27 Asp 329 A A 2.23 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou,. Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ala 330 A A 2.44 Glu 331 A A 2.43 His 332 A T 1.84 Tyr 333 A T 0.84 Asp 334 A T 0.03 Thr 335 A T 08 Ala 336 A A 39 Ile 337 A A 24 0.00 Leu 338 A B I 0 Phe 339 A B 0.00 Thr 340 A B 50 Arg 341 A B 58 03.

Gin 342 A T Asp 343 A T 0.48 Leu 344 A T 1. IS cys 345 T T 1.18 Gly 346 T T 0.40 Ser 347 T T 0.40 Gin 348 B T 0.09 Thr 349 B 0.09 cys 350 B 0.41 Asp 351 B T I f 0.16 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Thr 352 B T 13 Leu 353 B T 13 Gly 354 B T 68 Met 355 B 36 Ala 356 B 67 Asp 357 B T 21 Val 358 B T 07 Gly 359 B T 72 17i Thr 360 B T 33 I 0 Val 361 B 04 cys 362 B 0.07 Asp 363 B T 0.62 17@ Pro 364 T T 0.30 Ser 365 T T 0.31 I 5 Arg 366 T T 0.31 Ser 367 B T 0.09 cys 368 B B 0.09 Ser 369 B B 0.30 Val 370 B B 0.60 Ile 371 B B 0.14 Glu 372 B B 37 Asp 373 A T 0.30 I 1 - I - Res POS. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asp 374 A T 0.01 Gly 375 A T 0.28 Leu 376 A T 0.47 Gin 377 A A 0.16 Ala 378 A A 16 Ala 379 A A 74 Phe 380 A A 43 Thr 381 A A 0.38 17i Thr 382 A A 43 I 0 Ala 383 A A 19 His 384 A A 0.37 Glu 385 A A 0.21 Leu 386 A A 18 Gly 387 A B 0.13 0.12 His 388 A B Val 389 A B 06 Phe 390 A B 09 Asn 391 B B 0.72 Met 392 B T 1.07 Pro 393 A T 0.51 His 394 T T 1.41 Asp 395 A T 2.11 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asp 396 A A 1.44 Ala 397 A A 1.46 Lys 398 A A 1.37 Gin 399 A A 0.59 Cys 400 A B 0.59 Ala 401 A B 0.24 Ser 402 B T 02 Leu 403 B T 07 Asn 404 T T 07 Gly 405 T T 0.60 Val 406 c 0.89 Asn 407 T c 1.16 Gin 408 A 1.37 Asp 409 A T 0.77 Ser 410 A T 0.52 His 411 A A 1.08 Met 412 A A 0.48 Met 413 A A 33 Ala 414 A A 63 Ser 415 A A 33 Met 416 A A 11 Leu 417 A T 51 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ser 418 A T 0.06 Asn 419 A T 0.34 Leu 420 T c 0.64 Asp 421 T T 1.03 His 422 T T 1.56 Ser 423 T c 1.56 On 424 T C 1.34 Pro 425 1.49 Trp 426 T 1.19 Ser 427 T C 0.63 Pro 428 T T 0.69 Icys 429 T T 0.09 Ser 430 B T 59 Ala 431 B B 61 Tyr 432 B B 61 Met 433 B B 10 Ile 434 B B 24 Thr 435 B B 94 Ser 436 B B 36 Phe 437 B B 46 Leu 438 B T 0.11 Asp 439 T T 0.66 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asn 440 T c 0.97 Gly 441 T T 0.60 His 442 T T 0.49 Gly 443 A T 0.70 Glu 444 A T 0.70 Ln cys 445 B T 0.74 Leu 446 A B 0.88 Met 447 A B 0.91 17@ Asp 448 A T 1.26 I 0 Lys 449 A c 1.04 Pro 450 T T 0.82 GIn 451 T T 1.63 Asn 452 B T 1.42 Pro 453 B T 1.21 Ile 454 B 0.82 GIn 455 B 1.03 Leu 456 B T 0.22 Pro 457 B T 0.01 Gly 458 B T 12 Asp 459 B T 0.46 Leu 460 T c 0.16 Pro 461 B T 0.72 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gly 462 B T 0.93 Thr 463 B T 0.69 Ser 464 B 0.69 Tyr 465 T 1.61 Asp 466 T T 1.82 Ala 467 T T 1.50 Asn 468 T T 1.81 Arg 469 B T 1.41 Gin 470 B B 1.34 Cys 471 B B 0.64 Gin 472 B B 0.89 Phe 473 B B 0.89 (7i 0.78 Thr 474 B B Phe 475 B T 0.48 5 Gly 476 T T 1.19 Glu 477 T T 1.16 Asp 478 T T 1.19 Ser 479 T T 1.29 Lys 480 T 1.99 His 481 T 1.74 Ys 482 ro 483 A Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asp 484 T T 0.84 Ala 485 A T 0.13 Ala 486 A 13 Ser 487 B T T 0.22 Thr 488 B T 38 cys 489 B T 67 Ser 490 B T 74 Thr 491 B B 47 Leu 492 B B 51 Trp 493 B B 51 cys 494 B B 14 Thr 495 B B T 19 Gly 496 B T 22 Thr 497 T T 27 Ser 498 T T 79 Gly 499 T T 98 Gly 500 T T 33 Val 501 B B 99 Leu 502 B B 99 Val 503 B B 64 cys 504 B -0,33 Gln 505 B T 69 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Thr 506 B T 04 Lys 507 B T 0.48 His 508 C 0.74 Phe 509 B 1.41 Pro 510 T 1.07 Trp 511 T T 1.07 Ala 512 T T 0.72 Li ti Asp 513 T T 0.09 Gly 514 T T 0.44 I 0 Thr 5 1 5 T T 0.66 Ser 516 T T 0.60 cys 517 T T 1.23 Gly 518 T T 0.94 Glu 519 T 0.62 5 Gly 520 T 0.04 Lys 521 T 0.34 Trp 522 T 0.67 cys 523 B T 1.06 Ile 524 B T 0.39 Asn 525 T T 12 Gly 526 T 17 Lys 527 T 0.17 Res Pos. Garni.. Chou-... Garni.. Chou,. Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Cys 528 T T 0.52 Val 529 B T 1.41 Asn 530 B T 1.52 Lys 531 B T 1.91 Thr 532 B T 1.83 Asp 533 T T 1.80 Arg 534 T T 2.66 Lys 535 B T 2.34 His 536 B 2.09 Phe 537 B 1.70 Asp 538 B 1.67 Thr 539 B 1.21 Pro 540 c 0.87 Phe 541 T 0.61 5 His 542 T T 0.97 Gly 543 T T 0.37 Ser 544 T T 0.39 Trp 545 T T 0.26 Gly 546 c 0.74 Met 547 0.49 Trp 548 r 0.49 Gly 549 T c 0.79 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Pro 550 T T 0.41 Trp 551 T T 0.46 Gly 552 T T 1.17 Asp 553 T 1.14 cys 554 T T 0.82 c*1 !Z@ Ser 555 T T 0.69 Arg 556 T T 0.63 Thr 557 T T 0.63 I 0 cys 558 T T 22 Gly 559

T T 0.44 Gly 560 T T 0.50 Gly 561 T T 0.08 P tTI Val 562 B B 21 k1i NZ 1 5 Gln 563 B B 0.57 Tyr 564 B B 0.91 Thr 565 B B 0.59 Met 566 B B 0.93 Arg 567 B B 1.79 Glu 568 T 1.58 cys 569 T T 0.97 Asp 1 570 T T 1.07 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asn 571 T c 1.71 Pro 572 T c 1.60 Val 573 c 1.26 Pro 574 T T 1.58 Lys 575 T T 1.62 Asn 576 T T 1.38 Gly 577 T T 0.92 Gly 578 T T 1.78 Lys 579 B T 1.64 I 0 Tyr 580 B T 1.64 cys 581 B T 1.76 Glu 582 B 1.24 Gly 583 B B 1.70 Lys 584 B B 1.41 Arg 585 B B 1.77 Tyr 586 B B 2.13 Arg 587 B B 1.47 Tyr 588 B T 1.81 Arg 589 T T 0.96 Ser 590 T T 0.84 cys 591 T T 1.70 Asn 1 592 A T 0.92 Res Pos. Garni.. Chou-... Garni.. Chow... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Leu 593 A B 0.96 Glu 594 A B 0.84 Asp 595 A T 1.17 Cys 596 B T 1.81 Pro 597 T T 1.47 Asp 598 T T 2.32 Asn 599 T T 2.01 Asn 600 T T 1.31 Gly 601 T T 2.09 I 0 Lys 602 T c 2.30 Thr 603 T c 2.30 Phe 604 A A 2.30 Arg 605 A A 1.63 Glu 606 A A 1.98 Glu 607 A A 1.34 Gln 608 A A 1.62 cys 609 A A 2.32 Glu 610 A A 2.21 Ala 611 A A 1.51 His 612 A A 1.21 Asn 613 A A 1.26 Glu 614 _F A A 1.33 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Phe 615 A A 1.03 Ser 616 A A 0.92 Lys 617 A A 0.61 Ala 618 A T 0.31 Ser 619 A T 03 Phe 620 0.46 Gly 621 T T 0.17 Ser 622 T C 73 Gly 623 T c 14 Pro 624 T c 13 Ala 625 A c 32 tt Val 626 A B 19 Glu 627 A B 0.16 Trp 628 A B 0.26 lie 629 B 12 Pro 630 B T 0.12 Lys 631 T T 0.12 Tyr 632 T T 18 Ala 633 T T 10 Gly 634 T 0.83 Val 635 B 1.04 Ser 636 B T 1.11 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Pro 637 T T 0.69 Lys 638 T T 1.32 Asp 639 T T 0.86 Arg 640 A A 0.82 Cys 641 A A 0.46 Lys 642 A B 0.67 Leu 643 A B 0.03 1*4 lie 644 A B 0.08 Cys 645 A B 38 Gin 646 A B 60 Ala 647 A B 99 Lys 648 A B 42 Gly 649 T T 23 Ile 650 T T 27 1 5 Gly 651 B T 12 Tyr 652 B T 34 Phe 653 B B 39 Phe 654 B B 26 Val 655 B B 32 Leu 656 B B 83 Gin 657 B T 44 Pro 658 B T L 74 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Lys 659 T T 39 Val 660 B T 0.16 Val 661 B T 0.76 Asp 662 B 0.09 Gly 663 B T 0.00 Thr 664 B T 26 Pro 665 B 0.60 Cys 666 T 1.16 Ser 667 T c 0.84 PI I 0 Pro 668 T T 0.89 Asp 669 T T 0.34 Ser 670 T T 11 Thr 671 B T 30 Ser 672 B B 0.00 Val 673 B B 13 cys 674 B B 13 Val 675 B B 50 Gin 676 B 13 04 Gly 677 B B 70 Gin 678 B B 43 cys 679 B B 11 Val 680 B B 0.08 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Lys 681 B T 0.08 Ala 682 B T 0.53 Gly 683 B T 36 Cys 684 B T 58 Asp 685 A B 0.28 Arg 686 A B 07 lie 687 A B 0.57 0.96 Ile 688 A B Asp 689 A T 1.67 I 0 Ser 690 A T 0.97 Lys 691 A T 0.86 Lys 692 T T 1,79 Lys 693 T 2.01 Phe 694 F 1.67 1 5 Asp 695 T T 1.11 Lys 696 B T 0.40 cys 697 B T 0.01 Gly 698 B T 38 Val 699 B 0.32 Cys 700 T 02 Gly 701 T T 37 Gly 702 T T 01 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asn 703 T T 33 Gly 704 T T 0.57 Ser 705 T T 1.28 Thr 706 B T 0.73 cys 707 B T 0.78 Lys 708 B T 0.43 Lys 709 B 0.48 lie 710 B T 08 Ser 711 B T 08 I 0 Gly 712 B T 0.29 Ser 713 B T 34 Val 714 B B 34 Thr 715 B B 0.33 Ser 716 B B 0.29 Ala 717 B 0.39 Lys 718 T C 0.66 Pro 719 T T 1.51 Gly 720 T T 0.93 Tyr 721 B T 0.34 His 722 B B 0.62 Asp 23 B B 31 Ile 724 B B 31 T@* Res Pos. Garni.. Chou-... Garni.. Chow... Garni... Chow... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ile 725 B B 28 Thr 726 B B 38 Ile 727 B T 93 Pro 728 B T 24 Thr 729 T c 36 Gly 730 T c 36 R Ala 731 B c 04 Thr 732 B c 01 17i Asn 733 B B 0.24 Ile 734 B B 0.56 Glu 735 B B 1.01 Val 736 B B 1.60 Lys 737 B B 1.91 Gin 738 B 2.02 Arg 739 B 2.57 Asn 740 B T 2.27 Gin 741 T T 3.23 Arg 742 T T 3.19 Gly 743 T T 3.19 Ser 744 T 2.73 Arg 745 c 2.43 Asn 746 T T 1.73 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asn 747 T T 0.81 Gly 748 T c 0.57 Ser 749 B T 02 Phe 750 A B 09 Leu 751 A B 68 cn Ala 752 A B 27 5!z - Ile 753 A B 92 Lys 754 A A 97 Ala 755 A A 58 Ala 756 A A 401 Asp 757 A T 31 Gly 758 B T 23 Thr 759 B T 28 Tyr 760 B T 03 5 Ile 761 B 0.56 Leu 762 B 0.31 Asn 763 B T 0.34 Gly 764 T T 16 Asp 765 T T 21 Tyr 766 T c 0.37 Thr 767 B B 0.37 Leu 768 B B 0.37 Res Pos. Garni.. Chou-... Garni..

Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ser 769 B B 0.71 Thr 770 B B 0.71 Leu 771 A B O@07 Glu 772 A B 22 Gln 773 A B 0.34 co@ Asp 774 A B 0.69 Ile 775 A B 0.66 Met 776 A B 0.61 Tyr 777 B B 24 .cn 10 Lys 778 B B 06 Gly 779 B B 94 Val 780 B B 30 Val 781 B B 0.00 k@4 Leu 782 B B 10 Arg 783 B B 44 Tyr 784 B T 40 Ser 785 T T 13 Gly 786 T c 0.13 Ser 787 T c 0.13 Ser 788 A c 0.02 Ala 789 A A 0.38 Ala 790 A A 21 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Leu 791 A A 0.24 Glu 792 A A 0.24 Arg 793 A B B 16 Ile 794 A A B 0.13 Arg 795 A A B 0.51 Ser 796 A T 0.51 Phe 797 c 0.56 0.44 Ser 798 T c Pro 799 T c 1.12 I 0 Leu 800 T T 0.20 Lys 801 T C 0.19 Glu 802 c 0.00 Pro 803 A B 0.30 Leu 804 A B 34 1 5 Thr 805 B B 34 Ile 806 B B 70 Gln 807 B B 56 Val 808 B B 69 Leu 809 B B 88 Thr 810 B B 16 Val 811 B B 08 Gly 812 B B 97 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asn 813 A 32 Ala 814 A 0.53 Leu 815 A 04 Arg 816 B 0.86 Pro 817 B 0.96 Lys 818 B B 0.64 Ile 819 B B 0.99 Lys 820 B B 1.10 Tyr 821 B B 0.13 I O Thr 822 B B 0.39 Tyr 823 A B 0.39 Phe 824 A B 1.32 Val 825 A B 1.32 Lys 826 A B 1.57 1 5 Lys 827 A A 1.58 Lys 828 A A 1.12 Lys 829 A A 1.82 Glu 830 A A 2.09 Ser 831 A A 1.16 Phe 832 A A 0.90 Asn 833 A B 0.54 Ala 834 B 20 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ile 835 c 50 Pro 836 T c 79 Thr 837 T T 38 Phe 838 A T 23 Ser 839 T c 53 Ala 840 A B B 64 Trp 841 A B B 43 Val 842 A A B 41 Ile 843 A A B 06 I 0 Glu 844 A A B 0.24 Glu 845 A A 0.17 Trp 846 A A 0.16 Gly 847 A A 1.06 Glu 848 A T 1.64 cys 849 A T 0.98 Ser 850 T T 0.98 Lys 851 T T 0.46 Ser 852 T T 0.46 cys 853 T T 0.17 Glu 854 A A 0.83 Leu 855 A A 1.24 Gly 856 A 1.31 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou,. Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Bets Beta Turn Turn Coil Hydro... Alpha Beta Trp 857 A A 0.80 Gln 858 A A 0.61 Arg 859 A A 0.61 Arg 860 A B 0.76 Leu 861 A B 1.21 Lo @ Val 862 A B 1.50 Glu 863 A B 0.61 0.50 cys 864 A B Arg 865 A T 0.04 Asp 866 T T 0.86 lie 867 T T 1.50 Asn 868 T T 0.91 Gly 869 T c 1.28 Gln 870 T c 1.17 Pro 871 T c 0.50 Ala 872 T c 0.80 Ser 873 A T 0.84 Glu 874 A A 1.19 cys 875 A A 0.33 Ala 876 A A 0.59 Lys 877 A A 0.97 Glu 878 A A 0.68 Res POS. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Val 879 A A 0.38 Lys 880 A A 0.73 Pro 881 A T 1.43 Ala 882 T T 1.18 Ser 883 T T 0.51 Thr 884 T T 0.78 Arg 885 B T 0.73 Pro 886 T T 0.91 Cys 887 T T 1.29 I 0 Ala 888 T T 0.92 Asp 889 T 1.02 His 890 T C 0.91 Pro 891 T T 0.83 cys 892 T T 1.50 1 5 Pro 893 T T 1.28 Gin 894 A T 0.93 Trp 895 A B 0.97 Gin 896 A B 0.89 Leu 897 A B 1.26 Gly 898 T 1.17 Glu 899 T 0.50 Trp 900 T 0.49 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eis Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Bi Ser 901 T T 0.53 Ser 902 T T 1.03 Cys 903 T 'r 0.71 Ser 904 T T 0.37 Lys 905 T 0.70 Thr 906 T 0.66 17@ Cys 907 T 0.71 Gly 908 T T 1.42 Lys 909 T 1.77 I 0 Gly 910 T T 1.83 Tyr 911 T T 1.84 Lys 912 A B 1.70 Lys 913 A B 2.09 Arg 914 A B 1.38 1 5 Ser 915 A B 0.91 Leu 916 A B 0.86 Lys 917 A B 0.78 cys 918 A B 0.73 Leu 919 A B 0.28 Ser 920 B 0.23 His 921 B T 0.19 Asp 922 T I T 67 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gly 923 T T 30 Gly 924 T T 0.48 Val 925 B 0.78 Leu 926 B 0.51 Ser 927 B 16 His 928 B T 0.19 Glu 929 B T 0.32 Ser 930 A T 0.37 cys 931 A T 1.22 I 0 Asp 932 A T 1.57 Pro 933 A T 1.39 Leu 934 A T 1.43 Lys 935 A T 1.70 Lys 936 A A 1.67 Pro 937 A A 0.78 Lys 938 A A 0.99 His 939 A A 1.10 Phe 940 A B 0.39 Ile 941 A B 0.03 Asp 942 A A 36 Phe 943 A A 99 cys 944 A A 96 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni... Chou-... Garni.. Kyte-... Eisen.. Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Thr 945 A A 92 Met 946 A A 33 Ala 947 A A 72 Glu 948 A A 41 cys 949 A A 13 Ser 950 A A 21 17i Table 2 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Met I B 37 Phe 2 B 57 Pro 3 B 77 Ala 4 c 59 cn Pro 5 c 09 Ala 6 c 0.22 Ala 7 T c 0.11 lo Pro 8 A T 0.11 cv@ Arg 9 T T 0.00 Trp 10 B T 60 Leu 11 A B 82 Pro 12 A B 04 1 5 Phe 13 A B 64 Leu 14 A A 57 Leu 15 A A 09 Leu 16 A A -3,09 Leu 17 A A 69 Leu 18 A A 80 Leu 19 A A 20 Leu 20 A A 20 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Leu 21 A A 98 Leu 22 A B 06 Pro 23 A B 59 Leu 24 A A

37 Ala 25 A A 77 Arg 26 A B 54 Gly 27 A B 0.38 Ala 28 B 0.38 Pro 29 c 0.60 I 0 Ala 30 B 0.60 Arg 31 B 0.14 Pro 32 B 0.14 Ala 33 B T 0.73 Ala 34 A T 0.36 1 5 Gly 35 T c 0.64 Gly 36 T c 0.53 Gln 37 A 07 Ala 38 B 33 Ser 39 B B B 60 Glu 40 B B B 47 Leu 41 B B B 43 Val 42 B B B 32 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen.., Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Val 43 B B B 54 Pro 44 B B B 46 Thr 45 B B B 80 Arg 46 B B B 29 Leu 47 T C 02 C*n Pro 48 T C 0.49 Gly 49 T C 0.70 Ser 50 T c 0.20 Ala 5 1 A A 50 I 0 Gly 52 A A 50 Glu 53 A A 32 Leu 54 A A 79 Ala 55 A A 79 Leu 56 A A 79 His 57 A A 14 Leu 58 A A 49 Ser 59 A A 63 Ala 60 A A 39 Phe 61 A A 28 Gly 62 T T 10 Lys 63 A T 10 Gly B T 69 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Phe 65 B T 91 Val 66 B B 80 Leu 67 B B 67 Arg 68 B B 71 Leu 69 B B 37 Ala 70 T c 0.03 Pro 71 T c 0.19 Asp 72 T T 0.19 Asp 73 A T 51 I O Ser 74 A A 0.09 Phe 75 A A 0.68 Leu 76 A A 0.19 Ala 77 A A 0.23 Pro 78 A A 66 1 5 Glu 79 A A 36 Phe 80 A A OA6 Lys 81 A A 0.46 Ile 82 A A 0.70 Glu 83 A A 0.57 Arg 84 A A 0.27 Leu 85 A T 0.62 Gly 86 A T 0.69 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou,. Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gly 87 T C 0.99 Ser 88 T C 0.68 Gly 89 T c 0.22 Arg 90 B T 0.69 Ala 91 T C 1.03 cn Thr 92 B T 1.49 Gly 93 B T 1.44 Gly 94 T T 0.98 Glu 95 B 0.98 Arg 96 B 1.22 Gly 97 T 0.87 Leu 98 B T 0.51 Arg 99 B T 0.16 Gly 100 B T 14 5 cys 101 B T 60 Phe 102 B 57 Phe 103 B T 61 Ser 104 B T 72 Gly 105 T C 72 Thr 106 T C 06 Val 107 B c 0.43 Asn 108 B C 1.13 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou,.. Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gly 109 B C 1.13 Glu 110 T c 0.67 Pro III A T 0.39 Glu 112 A T 0.66 Ser 113 A T 20 Leu 114 A A B 16 Ala 115 A A B 97 A Ala 116 A B 42 Val 117 A A B 31 Ser 118 A B B 36 Leu 119 B B 36 Cys 120 B T 07 Arg 121 B T 82 Gly 122 T T 27 1 5 Leu 123 T T 67 Ser 124 T c 67 Gly 125 B T 81 Ser 126 B T 92 Phe 127 B T 92 Leu 128 A B C 11 Leu 129 A 0.19 Asp 130 A A 17 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gly 131 A A 18 Glu 132 A A 37 Glu 133 A A 0.44 Phe 134 A A 1.04 Thr 135 A B 1.04 lie 136 B 1.04 Gin 137 B 0.46 Pro 138 C 0.11 Gin 139 T 0.47 I 0 Gly 140 T C 0.48 Ala 141 T T 0.56 Gly 142 T c 03 Gly 143 T C 0.18 Ser 144 c 03 1 5 Leu 145 B 0.28 Ala 146 B 0.98 Gin 147 B T 0.51 Pro 148 B '17 0.86 His 149 B T 1.27 Arg 150 B T 1.79 Leu 151 B 2.03 Gin 152 B 1.82 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Arg 153 T 1.44 Trp 154 T 1.13 Gly 155 T C 0.43 Pro 156 T c 1.36 Ala 157 T T 1.14 Gly 158 T C 0.22 Ala 159 c 0.30 Arg 160 .13 0.76 Pro 161 B 0.62 I 0 Leu 162 c 1.00 Pro 163 C 1.34 Arg 164 C 1.64 1.53 Gly 165 T c Pro 166 T C 0.89 Glu 167 T C 1.70 Trp 168 A T 1.60 Glu 169 A 1.14 Val 170 A 1.49 Glu 171 A 1.36 Thr 172 A 1.36 Gly 173 T C 1.76 GM 174 A T 1.76 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou,. Garni.. Kyte-... Eisen... Eisen...

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gly 175 A T 2.61 Gin 176 A I T 2.72 Arg 177 A A 2.69 Gin A A 3.03 Glu 179 A T 3.00 Arg 180 A T 3.34 Gly 181 T T 3.34 Asp 182 T C 3.23 2.93 His 183 T c I 0 Gin 184 T C 2.93 Glu 185 A C 2.82 Asp 186 A 3.17 Ser 187 A A 2.87 Gin 188 A A 2.90 Glu 189 A A 2.90 Gin 190 A A 2.90 Ser 191 A A 2.90 Gin 192 A A 2.61 Gin 193 A A 2.61 Glu 194 A A 2.27 U 195 A A a 196 A A Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Glu 197 A A 1.68 Gly 198 A A 1.47 Ala 199 A T 1.26 Ser 200 C 1.04 Glu 201 C 1.42 Pro 202 c 0.61 Pro 203 c 0.61 Pro 204 T c 0.61 Pro 205 T c 0.60 I 0 Leu 206 T C 0.30 Gly 207 B T 0,62 Ala 208 B 0.52 Thr 209 B 0.78 k,j Ser 210 B T 1.10 Arg 211 B T 1.21 Thr 212 B T 0.70 Lys 213 B T 0.99 Arg 214 B 1.30 Phe 215 B B 1.01 Val 216 B B 1.01 Ser 217 A B 0.62 Glu 1 218 A A 28 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ala 219 A A B 39 Arg 220 A A B 0.00 Phe 221 A A B 0.04 Val 222 A A B 47 Glu 223 A A B 32 Lo@ Thr 224 A A B 32 Leu 225 A A B 43 Leu 226 A A B 32 17i A A B 77 Val 227 (0@ I 0 Ala 228 A A B 1.37 Asp 229 A A B 64 Ala 230 A A 42 Ser 231 A A 31 Met 232 A A 70 46 1 5 Ala 233 A A Ala 234 A A 04 Phe 235 A A 46 Tyr 236 A A 97 Gly 237 A A 37 Ala 238 A A 0.22 Asp 239 A A 0.78 Leu 240 A A 0.59 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen...

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gin 241 A A B 0.02 Asn 242 A A B 0.06 His 243 A B B 17 fie 244 A B B 77 Leu 245 A B B 26 Thr 246 A B B -I'll Leu 247 A B B 70 Met 248 A A 13 2.26 Ser 249 A A B 26 I 0 Val 250 A A B 33 Ala 251 A A B - 1.27 Ala 252 A A B 41 Arg 253 A A B 0.16 Ile 254 A A B 0.24 5 Tyr 255 A 0.80 Lys 256 B 0.50 His 257 B T 1.13 Pro 258 T c 1.02 Ser 259 T T 1.61 lie 260 T T 0.97 Lys 261 B 0.92 Asn 1 262 T 0.14 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-". Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ser 263 B B 24 Ile 264 B B 80 Asn 265 B B 77 Leu 266 B B 77 Met 267 A B 62 Val 268 B B -2,13 Val 269 B B 13 Lys 270 A B - 2.99 Val 271 B B 18 I 0 Leu 272 B B 58 Ile 273 A B 72 Val 274 A B 0.18 Glu 275 A B 16 0.36 Asp 276 A A 1 5 Glu 277 A A 0.96 Lys 278 A T 1.84 Trp 279 A c 1.84 Gly 280 T c 1.54 Pro 281 T c 1.54 Glu 282 B T 1.54 Val 283 B T 1.16 Ser 284 T c 1.10 Res Pos. Garni.. Chou-... Garni.. Chou,.. Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen.., Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asp 285 T T 0.63 Asn 286 T T 0.53 Gly 287 T T 28 Gly 288 T 0.69 Leu 289 B 0.99 Ln Thr 290 B 0.29 Leu 291 B 38 Arg 292 B 03 0.02 Asn 293 B I 0 Phe 294 T T 0.83 ys 295 T T 1.26 Asn 296 T T 2.18 17@ T 1.37 ,tft@ Trp 297 Gin 298 T 1.37 Arg 299 T 2.07 Arg 300 T 2.52 Phe 301 T 2.22 Asn 302 F 2.51 Gin 303 T c 2.62 Pro 304 T C 2.48 Ser 305 T T 2.16 Asp 306 T T 2.86 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Arg 307 c 2.82 His 308 c 2.58 Pro 309 c 2.79 On 310 T 2.78 His 311 A T 2.19 Tyr 312 A T 1.19 Asp 313 A T 0.41 Thr 314 A T 19 Ala 315 A B 50 I 0 Ile 316 B B 36 Leu 3 17 B B 11 Leu 318 B B 11 Thr 319 B B 50 Arg 320 B B 58 Gin 321 B T 03 Aso 322 T T 0.78 Phe 323 T T 1.59 Cys 324 T T 1.56 Gly 325 T T 0.63 Gln 326 T 03 Glu 327 T 03 Gly 328 T 0.36 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou,. Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Leu 329 B 0.21 Cys 330 B 0.21 Asp 331 B T 64 Thr 332 B T 23 Leu 333 B T 89 Ln Gly 334 B T 97 Val 335 B 64 Ala 336 B 96 Asp 337 B T 53 I 0 Ile 338 B T 39 Gly 339 B T 04 Thr 340 B T 40 Ile 341 B 0.19 Cys 342 B 0.23 5 Asp 343 B T 0.82 Pro 344 T T 0.50 Asn 345 T T 0.51 Lys 346 T T 0.54 Ser 347 B T 0.32 Cys 348 B B 0.32 Ser 349 B B 0.53 Val 1 350 B B 0.53 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ile 351 B B 0.14 Glu 352 A B 37 Asp 353 A A 0.30 Glu 354 A A 0.01 Gly 355 A A 0.28 Leu 356 A A 1.13 Gin 357 A A 0.82 Ala 358 A A 0.01 Ala 359 A A 58 I 0 His 360 A A 27 Thr 361 A A 0.54 Leu 362 A A 27 02 Ala 363 A A His 364 A A 0.53 1 5 Glu 365 A A 29 Leu 366 A A B 79 Gly 367 A A B 28 His 368 A A B 29 Val 369 A A B 47 Leu 370 A B B 50 Ser 371 A B 13 0.31 Met 372 B 0.66 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Pro 373 T 0.39 His 374 T T 1.29 Asp 375 T T 1.89 Asp 376 T T 1.52 Ser 377 T T 1.81 Lys 378 B T 2.13 Pro 379 T T 1.36 Cys 380 B T 0.66 Thr 381 B T 0.31 TO Arg 382 B B 0.40 Leu 383 B B 24 Phe 384 B B 38 Gly 385 B C 0.33 Pro 386 T c 0.61 Met 387 T T 0.47 Gly 388 A T 0.42 Lys 389 A T 0.52 His 390 A A 0.28 His 391 A A 0.28 Val 392 A B O@07 Met 393 A A 29 Ala 394 A A 19 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen.., Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Pro 395 A A 19 Leu 1 396 A I A 97 Phe 397 A A Val 398 A A 51 His 399 A B 23 Ln Leu 400 A B 83 Asn 401 A T 23 Gln 402 A T 0.18 Thr 403 A T 0.73 0.56 I 0 Leu 404 A C 0.70 Pro 405 T Trp 406 T 0.40 Ser 407 T C 19 Pro 408 T T 48 1 5 Cys 409 T T 0.09 Ser 410 B T 51 Ala 411 A B 53 Met 412 A B 23 Tyr 413 A B 83 Leu 414 A B 98 Thr 415 A B 68 A 43 Glu i 416 A Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Leu 417 A A 18 Leu 418 A T 0.03 Asp 419 T T 0.50 Gly 420 T T 0.81 Gly 421 T T 0.14 His 422 T T 0.14 Gly 423 T T 0.14 Asp 424 B T 0.14 Cys 425 B T 10 I 0 Leu 426 B 0.03 Leu 427 B 28 Asp 428 B 52 57i Ala 429 B T 11 tft@ ttl Pro 430 A T 26 Gly 431 T T 66 Ala 432 B T 66 Ala 433 B 87 Leu 434 B 59 Pro 435 B 72 Leu 436 B T 19 Pro 437 B T 81 Thr 1 438 T T 57 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gly 439 T c 0.36 Leu 440 T C 03 Pro 441 B T 0.19 Gly 442 B T 41 Arg 443 B T 34 Met 444 A B 0.00 Ala 445 A B 0.00 Leu 446 A B 0.21 Tyr 447 A B 0.56 I 0 Gin 448 A B 0.44 Leu 449 A A 0.38 Asp 450 A A 1.08 Gin 451 A B 1.89 I Gin 452 A B 1.24 5 Cys 453 A B 0.54 Arg 454 A B 1.01 Gin 455 A B 0.80 Ile 456 A B 0.80 Phe 457 A T 0.10 Gly 458 T c 0.88 Pro 459 T T 0.73 Asp 460 T T 0.07 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen...

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Phe 461 T T 0.74 Arg 462 T 1.44 His 463 T 1.48 Cys 464 T C 1.39 Pro 465 T T 0.80 Asn 466 T T 1.50 Thr 467 T T 1.39 0.57 Ser 468 A T Ala 469 A T 0.57 Gin 470 A B 0.19 Asp 471 A B 0.19 Val 472 A B 31 Cys 473 A B 30 Ala 474 A B 38 1 5 Gin 475 A B 41 Leu 476 A B 72 Trp 477 A B 0.13 Cys 478 A B 0.46 His 479 T T 0.46 Thr 480 T T 0.46 Asp 481 T T 1.06 Gly 482 T T 0.53 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen.., Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ala 483 T C 0.53 Glu 484 A 0.53 Pro 485 A 0.53 Leu 486 A 0.58 Cys 487 A 0.92 t;n His 488 B 1.17 Thr 489 T T 0.87 Lys 490 T T 0.27 T T 0.87 Asn 491 Gly 492 T T 1.24 Ser 493 c 0.69 Leu 494 C 1.00 Pro 495 B 0.61 Trp 496 T T 0.30 Ala 497 B T 0.43 Asp 498 T T 0.07 Gly 499 T T 0.53 Thr 500 T c 0.53 Pro 501 T T 0.48 Cys 502 T 1.03 Gly 503 T C 0.22 Pro 504 T 10 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen...

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gly 505 T 09 His 506 B 0.12 Leu 507 B 0.44 Cys 508 B T 0.49 Ser 509 T T 0.03 Glu 510 T T 43 Gly 511 T T 61 Ser 512 T 0.20 Cys 513 A c 0.87 I 0 Leu 514 A c 1.17 Pro 515

A A 0.31 Glu 516 A A 0.66 Glu 517 A A 1.07 Glu 518 A A 1.52 Val 519 A A 2.38 Glu 520 A A 2.38 Arg 521 A T 1.52 Pro 522 A T 0.67 Lys 523 A T 0.67 Pro 524 B T 1.18 Val 525 B 0.83 Val 526 B 0.43 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen...

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asp 527 B T 0.06 Gly 528 B T 20 Gly 529 T T 28 Trp 530 T C 0.23 Ala 531 C 0.88 Pro 532 T 0.59 Trp 533 T 0.59 Gly 534 T C 0.93 Pro 535 T T 0.56 I 0 Trp 536 T T 0.84 Gly 537 T C 1.17 Glu 538 T 1.14 Cys 539 T T 0.82 Ser 540 T T 0.69 Arg 541 T T 0,63 Thr 542 T T 0.63 Cys 543 T T 22 Gly 544 T T 0.44 Gly 545 T T 0.04 Gly 546 T T 37 Val 547 B B 09 Gin 548 B B I 0.69 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Phe 549 B B 1.03 Ser 550 B B 0.71 His 551 B 1.10 Arg 552 T 1.96 Glu 553 T 1.74 Ln Cys 554 T T 2.44 Lys 555 T T 2.53 Asp 556 T C 2.57 Pro 557 T c 2.46 I 0 Glu 558 C 2.11 Pro 559 T T 2.43 Gln 560 T T 2.50 T T 2.26 Asn 561 Gly 562 T T 1.80 ly 563 T T 0.99 Arg 564 B T 0.86 Tyr 565 B T 0.97 Cys 566 B T 1.08 Leu 567 B 0.83 Gly 568 B 1.22 Arg 569 B 0.87 Arg 1 570 T 1.11 I I Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ala 571 T 1.48 Lys 572 T 1.62 Tyr 573 T T 1.93 Gin 574 T T 1.51 Ser 575 T T 1.40 co@ Cys 576 T T 1.99 His 577 A B 1.28 Thr 578 A T 1.31 Glu 579 A T 1.10 I 0 Glu 580 A T 1.40 Cn Cys 581 A B 1.72 Pro 582 T C 1.80 Pro 583 T T 1.81 Asp 584 T T 1.11 1 5 Gly 585 T T 1.22 Lys 586 A T 1.89 Ser 587 A A 2.10 Phe 588 A A 2.31 Arg 589 A A 1.64 Glu 590 A A 1.99 Gin 591 A A 1.99 Gin 592 A A 2.04 Res Pos. Garni.. Chou-... Garni.. Chou,. Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen...

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Cys 593 A A 2.74 Glu 594 A T 2.04 Lys 595 A T 1.80 Tvr 596 T 1.80 Asn 597 T T 1.56 c'n Ala 598 T T 1.91 Tyr 599 B T 1.91 Asn 600 B T 1.27 Tyr 601 B 1.51 Thr 602 B 1.17 Asp 603 B T 1.76 @-q Met 604 B T 1.19 P Asp 605 T T 0.38 til Gly 606 B T 0.62 k @j NZ 1 5 Asn 607 B B 0.64 Len 608 A B 21 Leu 609 B B 0.18 Gin 610 B B 0.22 Trp 611 B B 0,32 Val 612 B B 27 Pro 613 B T 0.20 Res Pos. Garni.. Chou-... Garni.. Chou-_ Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Lys 614 B T 0.16 Tyr 615 B T 14 Ala 616 T T 07 Gly 617 T 0.90 Val 618 B 1.11 Ser 619 B T 1.18 Ln Pro 620 B T 0.76 Arg 621 T T 1.39 Asp 622 T T 0.92 I 0 Arg 623 A T 1.08 Cys 624 A B 0.71 Lys 625 A B 1.03 Leu 626 A B 0.33 Phe 627 A B 0.44 1 5 Cys 628 A B 01 Arg 629 A B 0.77 Ala 630 A T 0.42 Arg 631 T T 1,23 Gly 632 T T 1.23 Arg 633 T T 1.94 Ser 634 A 0.98 A Res Pos. Garni.. Chou-... Garni.. Chou,. Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Glu 635 A A 0.87 Phe 636 A A 0.76 Lys 637 A A 0.51 Val 638 A A 0.44 Phe 639 A A 11 Glu 640 A A 00 Ala 641 A B 30 Lys 642 A B 69 Val 643 A B 14 I 0 fie 644 A B 26 Asp 645 B B 92 Gly 646 B B 68 Thr 647 B B 93 Leu 648 B c 08 1 5 Cys 649 B T 0.50 Gly 650 T c 31 Pro 651 T T 56 Glu 652 A T 13 Thr 653 A T 99 Leu 654 A B 18 Ala 655 B B 72 Ile 656 B B 86 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Cys 657 B B 86 Val 658 A B 21 Arg 659 B B 26 Gly 660 B T T 62 Gin 661 B B 32 cys 662 B B 0.00 Val 663 B B 0.19 Lys 664 B T 0.08 Ala 665 B T 0.39 I 0 Gly 666 B T 47 Cys 667 B T 66 Asp 668 B B 0.20 His 669 B B 14 Val 670 B B 0.23 Val 671 B B 0.69 Asp 672 B B 1.40 Ser 673 B T 0.59 Pro 674 A T O@62 Arg 675 T T 1.52 Lys 676 T T 1.71 Leu 677 T 1.37 Asp 678 T T 0.81 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou,. Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Lys 679 B T 0.36 Cys 680 B T 10 Gly 681 B T 49 Val 682 B 0.37 Cys 683 B T 0 Gly 684 T T 02 T T 0.34 Gly 685 Lys 686 T T 0.02 Gly 687 T 0.99 Asn 688 T T 1.70 Ser 689 B T 1.19 Cys 690 B T 1.23 Arg 691 B T 0.84 Lys 692 B 0.89 1 5 Val 693 B T 0.08 Ser 694 B T 0.07 Gly 695 B T 0.52 Ser 696 B T 0.10 Leu 697 B 0.06 Thr 698 B 0.67 Pro 699 B T 0.62 Thr 700 T T 0.72 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen...

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Asn 701 B T 1.02 Tyr 702 T T 1.83 Gly 703 T T 1.26 Tyr 704 T T 0.61 Asn 705 B T 0.61 Asp 706 B T 28 lie 707 B B 24 Val 708 B B 49 17@ Thr 709 B B 59 Ile 710 B B 18 Pro 711 B T 49 Ala 712 B T 60 Gly 713 T C 63 Ala 714 T C 32 1 5 Thr 715 B B 29 Asn 716 B B 03 Ile 717 B B 0.56 Asp 718 B B 1.01 Val 719 B B 1.30 Lys 720 B B 1.58 Gln 721 B 1.37 Arg 1 722 B _F__F 1.91 Res Pos. Garni.. Chou-... Garni.. Chou,. Garni.. Chou,. Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Ser 723 c 1.06 724 T c 1.91 His Pro 725 T c 1.87 Gly 726 T T 1.87 Val 727 B T 1.41 Ln Gln 728 B 1.71 Asn 729 B T T 1.50 Asp 730 T T 0.90 Gly 731 T T 0.66 I 0 Asn 732 B

T 0.70 0.74 Tyr 733 A B Leu 734 A B 0.43 Ala 735 A B 16 Leu 736 A B 0.19 5 Lys 737 A B 16 Thr 738 B T 0.09 Ala 739 A T 0.66 Asp 740 B T 0.43 Gly 741 B T 0.43 Gln 742 B 0.39 rTyr 743 B 0.36 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Leu 744 B 0.94 Leu 745 B 0.13 Asn 746 B T 11 Gly 747 T T 00 Asn 748 T C 06 Leu 749 T c 83 co @ 17i Ala 750 A A B 91 B 91 Ile 751 A Ser 752 A B 57 Ala 753 A A 57 Ile 754 A A 64 Glu 755 A A 87 Gln 756 A B 83 Asp 757 A B 49 1 5 Ile 758 A B 24 Leu 759 A B 0.33 Val 760 A B 56 Lys 761 A B 37 Gly 762 B B 32 Thr 763 B B 68 Ile 764 B B 17 Leu 765 B B 0.34 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen.., Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Lys 766 B B 0.00 Tyr 767 B T 54 Ser 768 T c 82 Gly 769 T c 24 Ser 770 T C 24 Ile 771 A B 29 Ala 772 A 0.07 44 Thr 773 A B 10 (7i Leu 774 A B lo Glu 775 A A 10 Arg 776 A B 0.09 Leu 777 A T 0.79 Gin 778 A T 0.89 Ser 779 A T 0.89 5 Phe 780 B 0.68 Arg 781 c 0.57 Pro 782 c 1.17 Leu 783 T c 0.36 Pro 784 T c 0.34 Glu 785 T c 0.19 Pro 786 B T 0.08 Leu 787 B B 52 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen.., Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Thr 788 B B 52 Val 789 B B 62 Gln 790 B B 48 Leu 791 B B 48 Leu 792 B B 01 Thr 793 B B 70 Val 794 B T 70 Pro 795 B T 40 Gly 796 B T 80 I O Glu 797 B T 20 Val 798 B 0.16 Phe 799 B 0.16 Pro 800 B T 0.41 Pro 801 T T 0.51 1 5 Lys 802 T T 0.20 Val 803 B T 0.36 Lys 804 B B 0.36 Tyr 805 B B 29 Thr 806 B B 29 Phe 807 B B 33 Phe 808 B B 0.52 Val 809 B T Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Pro 810 B T 13 Asn 811 T T 52 Asp 812 T T 12 Val 813 A 02 Asp 814 A 0.83 Phe 815 A 0.74 Ser 816 A 0.44 Met 817 A 0.49 Gin 818 A T 1.34 lo Ser 819 T c 1.46 PI Ser 820 T C 1.57 Lys 821 A T 1.56 Glu 822 A 1.84 Arg 823 A B 1.84 NZ 15 Ala 824 A B 1.26 Thr 825 B B 0.67 Thr 826 B B 0.62 Asn 827 B B 0.41 Ile 828 B B 51 Ile 829 B B 73 Gin 830 A B 46 Pro 831 A B 73 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen..

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Leu 832 A B 73 Leu 833 A B 13 His 834 A B 10 Ala 835 A B B 91 Gln 836 A B B 04 Trp 837 A B B 23 Val 838 A B B 0.29 Leu 839 B T 0.02 Gly 840 T T 0.61 I O Asp 841 T T 06 Trp 842 T T 07 Ser 843 T c 0.49 Glu 844 T T 0.99 Cys 845 T T 0.67 1 5 Ser 846 T T 0.32 Ser 847 T 0.02 Thr 848 T 02 Cys 849 T 31 Gly 850 T T 0.36 Ala 851 T T 0.77 Gly 852 T T Trp Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-... Garni.. Kyte-... Eisen... Eisen...

Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Gln 854 B B 0.99 Arg 855 B B 1.33 Arg 856 B B 1.26 Thr 857 B B 1.71 Val 858 B B 2.00 Glu 859 B B 1.79 Cys 860 T 1.38 T 0.92 Arg 861 Asp 862 T C 1.23 I 0 Pro 863 T T 1.50 Ser 864 T T 1.20 Gly 865 T T 1.28 Gln 866 A 0.86 Ala 867 B 0.19 1 5 Ser 868 B 0.40 Ala 869 A 0.74 Thr 870 A T 0.50 Cys 871 A T 31 Asn 872 A T 0.32 Lys 873 A T 0.41 Ala 874 A 1.00 [@: 875 A 1.31 Res Pos. Garni.. Chou-... Garni.. Chou-... Garni.. Chou-..,. Garni.. Kyte-... Eisen... Eisen Alpha Alpha Beta Beta Turn Turn Coil Hydro... Alpha Beta Lys 876 A T 1.39 Pro 877 A T 1.43 Glu 878 A T 1.18 Asp 879 A T 1.10 Ala 880 A 1.91 Lys 881 A T 1.57 Pro 882 A T 1.78 Cys 883 A T 0.97 Glu 884 A T 0.30 I 0 Ser 885 A A 0.68 Gln 886 A B 18 Leu 887 A B 36 Cys 888 A B 08 Pro 889 A B 47 890 B 56 __F Leu Detailed Description By screening cDNA libraries with cDNA encoding the anti-angiogenic domain of TSP-1, the present inventors have identified two novel proteins, METHI and METH2 (also called VEGA-1 and VEGA-2, respectively, for vascular endothelial growth antagonist) which contain the anti-angiogenic domain of TSP- 1, a metalloproteinase domain, and a disintegrin-like domain. The present inventors have demonstrated that both METH I and METH2 have anti-angiogenic activity.

Thus, the present invention provides isolated nucleic acid molecules 1 0 comprising a polynucleotide encoding a METHI polypeptide having the amino acid sequence shown in SEQ ID NO: 2, which was determined by sequencing a cloned cDNA. The METHI protein of the present invention shares sequence homology with thrombospondin- I and pNPI. The nucleotide sequence shown in SEQ ID NO: I was obtained by sequencing a cDNA clone, which was deposited on January 15, 1998 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and given accession number 20958 1.

The cDNA clone contained in ATCC Deposit No. 209581 contains a METHI sequence, encoding amino acids I to 950 of SEQ ID NO:2.

The present invention also provides isolated nucleic acid molecules comprising a polynucleotide encoding a METH2 polypeptide having the amino acid sequence shown in SEQ ID NOA, which was partially determined by sequencing a

cloned cDNA. The METH2 protein of the present invention shares sequence homology with thrombospondin- I and pNPI. The nucleotide sequence shown in SEQ ID NO:3 was partially obtained by sequencing a cDNA clone, which was deposited on January 15, 1998 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209, and given accession number 209582. The cDNA clone contained in ATCC Deposit No. 2095 82 contains a partial METH2 sequence, encoding amino acids 1 12-890 of SEQ ID NOA.

Nucleic Acid Molecules Some of the nucleotide sequences determined by sequencing a DNA molecule herein were determined using an automated DNA sequencer (such as the Model 373 from Applied Biosystems, Inc.), and all amino acid sequences of polypeptides encoded by DNA molecules determined herein were predicted by translation of a DNA sequence determined as above. Therefore, as is known in the art for any DNA sequence determined by this automated approach, any nucleotide sequence determined herein may contain some errors. Nucleotide sequences determined by automation are typically at least about 90% identical, more typically at least about 95% to at least about 99.9% identical to the actual nucleotide sequence of the sequenced DNA molecule. The actual sequence can be more precisely determined by other approaches including manual DNA sequencing methods well known in the art. As is also known in the art, a single insertion or deletion-in a determined nucleotide sequence compared to the actual sequence will cause a frame shift in translation of the nucleotide sequence such that the predicted amino acid sequence encoded by a determined nucleotide sequence will be completely different from the amino acid sequence actually encoded by the sequenced DNA molecule, beginning at the point of such an insertion or deletion.

Using the information provided herein, such as the nucleotide sequence in SEQ ID NO: I or SEQ ID NO:3, a nucleic acid molecule of the present invention encoding a METHI or METH2 polypeptide may be obtained using standard cloning and screening procedures, such as those for cloning cDNAs using mRNA as starting material. Illustrative of the invention, the nucleic acid molecule described in SEQ ID NO: I was discovered in a cDNA library derived from human heart and the nucleic acid molecule described in SEQ ID NO:3 was discovered in a cDNA library derived from human lung. The determined nucleotide sequence of the METH I cDNA of SEQ ID NO: I contains an open reading frame encoding a protein of about 950 amino acid residues, including a predicted leader sequence of about 28 amino acid residues. The present inventors have determined that the nucleotide sequence of the METH2 cDNA of SEQ ID NO-3 contains an open reading frame encoding a protein of about 890 amino acid residues, including a predicted leader sequence of about 23 amino acid residues.

The present invention also provides the mature form(s) ofthe METH I and METI-12 proteins of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Most mammalian cells and even insect cells cleave secreted proteins with the same specificity. However, in some cases, cleavage of a secreted protein is not entirely uniform, which results in two or more mature species on the protein. Further, it has long been known that the cleavage specificity of a secreted protein is ultimately determined by the primary structure of the complete protein, that is, it is inherent in the amino acid sequence of the polypeptide. Therefore, the present invention provides a nucleotide sequence encoding the mature METHI polypeptide having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. 209581 and as shown in SEQ ID NO:2. The present invention also provides a nucleotide sequence encoding the mature METH2 polypeptide having the amino acid sequence as shown in SEQ ID NOA. By the mature METHI protein having the amino acid sequence encoded by the cDNA clone contained in the host identified as ATCC Deposit No. 209581 is meant the mature form(s) of the METHI protein produced by expression in a mammalian cell (e.g., COS cells, as described below) of the complete open reading frame encoded by the human DNA sequence of the clone contained in the vector in the deposited host. As indicated below, the mature METHI having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209581 may or may not differ from the predicted "mature" METH I protein shown in SEQ ID NO:2 (amino acids from about 29 to about 950) depending on the accuracy of the predicted cleavage site based on computer analysis; and the mature METH2 may or may not differ from the predicted "mature" METI-12 protein shown in SEQ ID NO: 4 (amino acids from about 24 to about 890) depending on the accuracy of the predicted cleavage site based on computer analysis.

Methods for predicting whether a protein has a secretory leader as well as the cleavage point for that leader sequence are available. For instance, the methods of McGeoch (Virus Res. 3:271-286 (1985)) and von Heinje (Nucleic Acids Res. 14:4683-4690 (1986)) can be used. The accuracy of predicting the cleavage points of known mammalian secretory proteins for each ofthese methods 0 is in the range of 75-80%. von Heinje, supra. However, the two methods do not always produce the same predicted cleavage point(s) for a given protein.

In the present case, the predicted amino acid sequence of the complete METHI and METH2 polypeptides of the present invention were analyzed by a computer program ("PSORT") (K. Nakai and M. Kanehisa, Genomics 14:897-911 (I 992)), which is an expert system for predicting the cellular location of a protein based on the amino acid sequence. As part of this computational prediction of localization, the methods of McGeoch and von Hemje are incorporated. The analysis by the PSORT program predicted the cleavage site between amino acids 28 and 29 in SEQ ID NO:2 and amino acids 23 and 24 in SEQ ID NOA, Thereafter, the complete amino acid sequences were fiirther analyzed by visual inspection, applying a simple form of the (-I,-3) rule of von Heinje. von Heinje, supra. Thus, the leader sequence for the METH I protein is predicted to consist of amino acid residues from about I to about 28 in SEQ ID NO:2, while the mature METH I protein is predicted to consist of residues from about 29 to about 950- and the leader sequence for the METI-12 protein is predicted to consist of amino acid residues from about I to about 23 in SEQ ID NOA, while the mature METI-12 protein is predicted to consist of residues from about 24 to about 890.

An alternative predicted mature METH I protein consists of residues 30 to 950 in SEQ ID NO:2.

As one of ordinary skill would appreciate, due to the possibilities of sequencing errors, as well as the variability of cleavage sites for leaders in different known proteins, the predicted METHI polypeptide encoded by the deposited cDNA comprises about 950 amino acids, but may be anywhere in the range of 910-990 amino acids- and the predicted leader sequence of this protein is about 28 amino acids, but may be anywhere in the range of about 18 to about 3 8 amino acids. Also, the predicted METH2 polypeptide comprises about 890 amino acids, but may be anywhere in the range of 850 to about 930 amino acids; and the predicted leader sequence of this protein is about 23 amino acids, but may be anywhere in the range of about 13 to about 33 amino acids.

As indicated, nucleic acid molecules of the present invention may be in the form of RNA, such as mRNA, or in the form of DNA, including, for instance, cDNA and genomic DNA obtained by cloning or produced synthetically. The DNA may be double-stranded or single-stranded. Single-stranded DNA or RNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also referred to as the anti-sense strand.

By "isolated" nucleic acid molecule(s) is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, recombinant DNA molecules contained in a vector are considered isolated for the purposes of the present invention. Further examples of isolated DNA molecules include recombinant DNA molecules maintained in heterologous host cells or purified (partially or substantially) DNA molecules in solution.

Isolated RNA molecules include in vivo or in vitro RNA transcripts of the DNA molecules of the present invention. Isolated nucleic acid molecules according to the present invention further include such molecules produced synthetically.

Isolated nucleic acid molecules of the present invention include DNA molecules comprising an open reading frame (ORF) shown in SEQ ID NO: II DNA molecules comprising the coding sequence for the mature METHI protein-, and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the NMTHI protein. Also included are DNA molecules comprising an open reading frame (ORF) shown in SEQ ID NO: 3 -) DNA molecules comprising the coding sequence for the mature NMTH2 protein; and DNA molecules which comprise a sequence substantially different from those described above but which, due to the degeneracy of the genetic code, still encode the NMTH2 protein. Of course, the genetic code is well known in the art. Thus, it would be routine for one skilled in the art to generate such degenerate variants.

In another aspect, the invention provides isolated nucleic acid molecules encoding the METH I or NVETH2 polypeptides having an amino acid sequence as I 0 encoded by the cDNA clones contained in the plasmids deposited as ATCC Deposit No. 209581 on January 15, 1998 or ATCC Deposit No. 209582 on January 15, 1998, respectively. In a further embodiment, nucleic acid molecules are provided encoding the mature WTHI or NffiTH2 polypeptide or the full- length METH I or METH2 polypeptide lacking the N-terminal methionine. The invention also provides an isolated nucleic acid molecule having the nucleotide sequence shown in SEQ ID NO: I or SEQ ID NO:3 or the nucleotide sequence of the METH I or MIETH2 cDNA contained in the above-described deposited clones, or a nucleic acid molecule having a sequence complementary to one of the above sequences. Such isolated molecules, particularly DNA molecules, are useful as probes for gene mapping, by in situ hybridization with chromosomes, and for detecting expression of the WTHI or NffiTH2 gene in human tissue, for instance, by Northern blot analysis.

The present invention is further directed to fragments of the isolated nucleic acid molecules described herein. By a fragment of an isolated nucleic acid molecule having the nucleotide sequence ofthe deposited cDNA or the nucleotide sequence shown in SEQ ID NO: I or SEQ ID NO: 3 is intended fragments at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length which are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550 @ 600@ 650, 700@ 750, 800, 850@ 900@ 950@ 1 000@ 10507 1 1 00@ 1200, 1300 @ 1400, 1500, 1600@ 1700@ 1800@ 1900@ 2000@ 2100, 2200@ 2300@ 2400@ 2500, 2600 @ 2700, 2800, 2900, or 3000 nt in length are also useful according to the present invention as are fragments corresponding to most, if not all, of the nucleotide sequence of the deposited cDNA or as shown in SEQ ID NO: I or SEQ ID NO:3. By a fragment at least 20 nt in length, for example, is intended fragments which include 20 or more contiguous bases from the nucleotide sequence of the deposited cDNA or the nucleotide sequence as shown in SEQ ID NO: I or SEQ ID NO: 3.

Preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding epitope-bearing portions of the METHI or METH2 protein. Methods for determining epitope-bearing portions of the METHI and WTH2 proteins are described in detail below.

Other preferred nucleic acid fragments of the present invention include nucleic acid molecules encoding: the metalloprotease domain of METH I, amino acids 235 to 459 in SEQ ID NO:2; the disintegrin domain of METHI, amino acids 460 to 544 in SEQ ID NO:2; the first TSP-like domain of METHI, amino acid s 5 4 5 to 5 9 8 in SEQ ID NO: 2the second T SP-like domain of METH 1, amino acids 841 to 894 in SEQ ID NO-2; the third TSP-like domain of METHI, amino acids 895 to 934 in SEQ ID NO:2; amino acids 536 to 613 in SEQ ID NO:2; amino acids 549 to 563 in SEQ ID NO:2; the metalloprotease domain of METH2, amino acids 214 to 439 in SEQ ID NOA the disintegrin domain of METH2, amino acids 440 to 529 in SEQ ID NOA; the first TSP-like domain of METH2, amino acids 530 to 583 in SEQ ID NOA; the second TSP-like domain of METH2, amino acids 837 to 890 in SEQ ID NOA amino acids 280 to 606 in SEQ ID NOA and amino acids 529 to 548 in SEQ ID NOA In addition, the present inventors have identified the following cDNA clones related to portions of the sequence shown in SEQ ID NO: 1: HOUCQ I 7RA (SEQ ID NO: 14), HPLBM I I R (SEQ ID NO: 15), HGBI07R (SEQ ID NO: 16), HNTMA49R (SEQ ID NO: 17), HNALE27R (SEQ ID NO: 18), and HIBDB45R (SEQ ID NO: 19).

The following public ESTs, which relate to portions of SEQ ID NO: 1, have also been identified: D67076 (SEQ ID NO:20), ABOO1735 (SEQ ID NO:21), X14787 (SEQ ID NO:22), U64857 (SEQ ID NO:23), X04665 (SEQ ID NO:24), M64866 (SEQ ID NO:25), L07803 (SEQ ID NO:26), U08006 (SEQ ID NO:27), MI 6974 (SEQ ID NO:28), LI 3 855 (SEQ ID NO:29), AL021529 (SEQ ID NO:30), D86074 (SEQ ID NO:3 1), L05390 (SEQ ID NO:32), Z69361 (SEQ ID NO:33), X99599 (SEQ ID NO:34), AF018073 (SEQ ID NO:35), L23760 (SEQ ID NO:36), Z46970 (SEQ ID NO:37), AC004449 (SEQ ID NO:38), Z69589 (SEQ ID NO:39), Z22279 (SEQ ID NOAO), and X17524 (SEQ ID NOAI).

The present inventors have also identified the following cDNA clones related to portions of SEQ ID NO:3: HCE4D69FP02 (SEQ ID NO:42), 1-UBDB45F (SEQ ID NO:43), HKIXH64R (SEQ ID NO:44), HIBDB45R (SEQ ID NO:19), HCE3Z95R (SEQ ID NO:45), HTLEQ90R (SEQ ID NO:46), HNIWEF45R (SEQ ID NO:47), HTOFC34RA (SEQ ID NO:48), HETD12OR (SEQIDNO:49),ITMSHY47R(SEQIDNO:50),HCESF90R(SEQIDNO:51), HMCA046R (SEQ ID NO:52), HTTAQ67R (SEQ ID NO:53), HIFKCF19F (SEQ ID NO:54), HMCAS31R (SEQ ID NO:55), HNffGP26R (SEQ ID NO:56), HLHTP36R (SEQ ID NO:57), HE8ANIIR (SEQ ID NO:58), HEONN73R(SEQIDNO:59),HBNBG53R(SEQIDNO:60),andl-IMSCH94R (SEQ ID NO: 61).

The following public ESTs, which relate to portions of the sequence shown in SEQ ID NO:3, have also been identified: D67076 (SEQ ID NO:20), ABOO1735 (SEQ ID NO:21), ABOO5287 (SEQ ID NO:62), X87619 (SEQ ID NO:63), X14787 (SEQ ID NO:22), X04665 (SEQ ID NO:24), M87276 (SEQ ID NO:64), M62458 (SEQ ID NO:65), ABOO2364 (SEQ ID NO:66), ABOO5297 (SEQ ID NO:67), X69161 (SEQ ID NO:68), XI 6619 (SEQ ID NO:69), I36448 (SEQ ID NO:70), L12260 (SEQ ID NO:71),136352 (SEQ ID NO:72), X15898 (SEQ ID NO:73),107789 (SEQ ID NO:74),108144 (SEQ ID NO:75) U31814 (SEQ ID NO:76), and AF001444 (SEQ ID NO:77).

In specific embodiments, the polynucleotides of the invention are less than 300 kbl 200 kbl 100 kb@ 50 kb@ 15 kbl

10 kb, or 7.5 kb in length. In a further embodiment, polynucleotides of the invention comprise at least 15 contiguous nucleotides of METHI or METH2 coding sequence, but do not comprise all or a portion of any METH I or METI-12 intron. In another embodiment, the nucleic acid comprising METHI or NETI-12 coding sequence does not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the NMTHI or METH2 gene in the genome).

In another aspect, the invention provides an isolated nucleic acid molecule 1 0 comprising a polynucleotide which hybridizes under stringent hybridization conditions to a portion of the polynucleotide in a nucleic acid molecule of the invention described above, for instance, the cDNA clones contained in ATCC Deposit No. 209581 or ATCC Deposit No. 209582. By "stringent hybridization conditions" is intended overnight incubation at 42'C in a solution comprising.

t5 50% formamide, 5x SSC (750 mM NaCl, 75mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 [tg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0. Ix SSC at about 65'C.

By a polynucleotide which hybridizes to a "portion" of a polynucleotide is intended a polynucleotide (either DNA or RNA) hybridizing to at least about 15 nucleotides (nt), and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably about 30, 40, 50, 60 or 70 nt of the reference polynucleotide. These are useful as diagnostic probes and primers as discussed above and in more detail below.

By a portion of a polynucleotide of "at least 20 nt in length," for example, is intended 20 or more contiguous nucleotides from the nucleotide sequence ofthe reference polynucleotide (e.g., the deposited cDNAs or the nucleotide sequence as shown in SEQ ID NO: I or SEQ ID NO: 3). Of course, a polynucleotide which hybridizes only to a poly A sequence (such as the 3' terminal poly(A) tract of the METHI or METH2 cDNA shown in SEQ ID NO-.1 and SEQ ID NO:3 @ - respectively) or to a complementary stretch of T (or U) resides, would not be included in a polynucleotide of the invention used to hybridize to a portion of a nucleic acid of the invention, since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

Also contemplated are nucleic acid molecules that hybridize to the NMTHI or METH2 polynucleotides at moderately high stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, moderately high stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl- 0.2M NaH2PO4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, I 00 [tg/ml salmon sperm blocking DNA; followed by washes at 5 O'C with I XS SPE, 0. I% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSQ.

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any Yterminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide, " since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone).

The METHI or METH2 polynucleotide can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, METHI or METH2 polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single-and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single-and double-stranded regions. In addition, the METHI or METH2 polynucleotides can be composed of triple- stranded regions comprising RNA or DNA or both RNA and DNA. METH I or METH2 polynucleotides may also contain one or more modified bases or DNA or RNA backbones modified for stability or for other reasons. "Modified" bases

include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

"SEQ ID NO: I " refers to a METHI polynucleotide sequence while " SEQ ID NO:2" refers to a METHI polypeptide sequence. "SEQ ID NO:3" refers to a METH2 polynucleotide sequence while "SEQ ID NOA? refers to a METH2 polypeptide sequence.

As indicated, nucleic acid molecules of the present invention which encode a METHI or METH2 polypeptide may include, but are not limited to, those encoding the amino acid sequence of the mature polypeptide, by itself, the coding sequence for the mature polypeptide and additional sequences, such as those encoding the leader or secretory sequence, such as a pre-, or pro- or prepro-protein sequence- the coding sequence of the mature polypeptide, with or without the aforementioned additional coding sequences, together with additional, non-coding sequences, including for example, but not limited to introns and non-coding 5' and 3' sequences, such as the transcribed, non-translated sequences that play a role in transcription, mRNA processing, including splicing and polyadenylation signals, for example - ribosome binding and stability of mRNA; an additional coding sequence which codes for additional amino acids, such as those which provide additional functionalities. Thus, the sequence encoding the Polypeptide may be fused to a marker sequence, such as a sequence encoding a peptidewhichfacilitatespurificationofthe. Incertainpreferred embodiments of this aspect of the invention, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (Qlagen, Inc.), among others, many of which are commercially available. As described in Gentz el aL, Proc. NaIL Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. The "HA" tag is another peptide useful for purification which corresponds to an epitope derived from the influenza hemagglutinin protein, which has been described by Wilson el aL, Cell 37:767-778 (1984). As discussed below, other such fusion proteins include the NIETHI or METH2 fused to Fc at the N- or C-terminus.

The present invention further relates to variants of the nucleic acid molecules of the present invention, which encode portions, analogs or derivatives of the METH I or METH2 protein. Variants may occur naturally, such as a natural allelic variant. By an "allelic variant" is intended one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. Lewin, B., ed., Genes H, John Wiley & Sons, New York (1985). Non-naturally occurring variants may be produced using art-known mutagenesis techniques.

Such variants include those produced by nucleotide substitutions, deletions or additions, which may involve one or more nucleotides. The variants may be altered in coding regions, non-coding regions, or both. Alterations in the coding regions may produce conservative or non-conservative amino acid substitutions, deletions or additions. Especially preferred among these are silent substitutions, additions and deletions, which do not alter the properties and activities of the METH I or METH2 protein or portions thereof Also especially preferred in this regard are conservative substitutions.

Further embodiments of the invention include isolated nucleic acid molecules comprising a polynucleotide having a nucleotide sequence at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical to: a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2- a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NO:2, but lacking the N-terminal methionine; a nucleotide sequence encoding the polypeptide having the amino acid sequence at positions from about 29 to about 950 in SEQ ID NO:2; a nucleotide sequence encoding the polypeptide having the amino acid sequence at position from about 30 to about 950 in SEQ ID NO:2; a nucleofide sequence encoding the polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209581- a nucleotide sequence encoding the mature NMTH1 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 20958 1; a nucleofide sequence encoding amino acids 235 to 459 in SEQ ID NO:2 (the metalloprotease domain of METHI); a nucleotide sequence encoding amino acids 460 to 544 in SEQ ID NO-.2 (the disintegrin domain of METHI)- a nucleotide sequence encoding amino acids 545 to 598 in SEQ ID NO:2 (the first TSP-like domain of N/IETHI); a nucleotide sequence encoding amino acids 841 to 894 in SEQ ID NO:2 (the second TSP-like domain of METHI); a nucleotide sequence encoding amino acids 895 to 934 in SEQ ID NO:2 (the third TSP-like domain of METHI)- a nucleotide sequence encoding amino acids 536 to 613 in SEQ ID NO:2- a nucleotide sequence encoding amino acids 549 to 563 in SEQ ID NO:2; a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NOA; a nucleotide sequence encoding the polypeptide having the amino acid sequence in SEQ ID NOA, but lacking the N-terminal methionine; a nucleotide sequence encoding the polypeptide having the amino acid sequence at positions from about 24 to about 890 in SEQ ID NOA a nucleotide sequence

encoding the polypeptide having the amino acid sequence at positions from about Il 2 to about 890 in SEQ ID NOA; a nucleotide sequence encoding the polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209582-5 a nucleotide sequence encoding the mature MIETH2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No, 209582; a nucleotide sequence encoding amino acids 214 to 439 in SEQ ID NOA (the metalloprotease domain of NTETI-12); a nucleotide sequence encoding amino acids 440 to 529 in SEQ ID NOA (the disintegrin domain of METI-12); a nucleotide sequence encoding amino acids 530 to 583 in SEQ ID NOA (the first TSP-like domain of METH2)- a nucleotide sequence encoding amino acids 837 to 890 in SEQ ID NOA (the second TSP-like domain of METH2); a nucleotide sequence encoding amino acids 280 to 606 in SEQ ID NO-4; a nucleotide sequence encoding amino acids 529 to 548 in SEQ ID NOA; or a nucleotide sequence complementary to any of the above nucleotide sequences .

By a polynucleotide having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence encoding a NMTHI or METH2 polypeptide is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide 5 sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the WTHI or NMTH2 polypeptide. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the referenc esequence may be inserted into the reference sequence. These mutations of the reference sequence may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence of in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular nucleic acid molecule is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the nucleotide sequence shown in SEQ ID NO: I or SEQ ID NO: 3 or to the nucleotide sequence of the deposited cDNA clones can be determined conventionally using known computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 5371 1). Bestfit uses the local homology algorithm of Smith and Waterman, Advances in AppliedMathematics 2: 482-489 (I 98 1), to find the best segment of homology between two sequences. When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference nucleotide sequence and that gaps in homology of up to 5% of the total number of nucleotides in the reference sequence are allowed.

A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al., Comp. Appl. Biosci.

6:237-245 (1990). In a sequence alignment, the query and subject sequences are both DNA sequences. An RNA sequence can be compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identity are: Matrix=Unitary, k-tuple=4, Mismatch Penalty= 1, Joining Penalty =3 0, Randomization Group Length=O, Cutoff Score=l, Gap Penalty=5, Gap Size Penalty=0.05,WindowSize=500rthelengthofthesubjectnucleotidesequ, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5'and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5'or Yends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5'and Tofthe subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by the results of the FASTDB sequence alignment.

This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final identity score. This corrected score is what is used for the purposes of the percenti presentinvention. Onlybasesoutsidethe5'and3'basesofthesubj, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence are calculated for the purposes of manually adjusting the percent

identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and, therefore, the FASTDB alignment does not show a match/alignment of the first IO bases at the 5' end. The I 0 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence), so IO% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a I 00 base query sequence. This time the deletions are internal, so that there are no bases on the 5'or Yends of the subject sequence which are not matched/aligned with the query.

In this case, the percent identity calculated by FASTDB is not manually corrected.

One again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The present application is directed to nucleic acid molecules at least 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in SEQ ID NO: I or SEQ ID NO:3 or to the nucleic acid sequence of the deposited cDNAs, irrespective of whether they encode a polypeptide having NIETHI or N11ETH2 activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having NEETHI or METH2 activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having NIETH I or METH2 activity include, inter alia, (1) isolating the NETH I or NIETH2 gene or allelic variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the METHI or METH2 gene, as described in Verma el al., Human Chromosomes: A Manual ofBasic Techniques, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting WTHI of METH2 mRNA expression in specific tissues.

1 0 Preferred, however, are nucleic acid molecules having sequences at least 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequence shown in SEQ ID NO: I or SEQ ID NO:3 or to a nucleic acid sequence of the deposited cDNAs which do, in fact, encode a polypeptide having METHI or NMTH2 protein activity. By "a polypeptide having METHI activity" is intended polypeptides exhibiting METHI activity in a particular biological assay. For example, METHI protein activity can be measured using the chorioallantoic membrane assay (Iruela-Arispe el al., Thrombosis and Haemostasis 78(i):672- 677 (1997)) or the cornea pocket assay (Tolsina et al., J Cell. Biol. 122:497-5 1 1 (I 993)), both described in Example 4, below. By "a polypeptide having METH2 activity" is intended polypeptides exhibiting METH2 activity in a particular biological assay. For example, METH2 protein activity can also be measured using the chorioallantoic membrane assay (Iruela-Arispe et al., Thrombosis and Haemostasis 78(l):672-677 (1997)) or the cornea pocket assay (Tolsma et al., J Cell. Biol. 122:497-511 (1993)), both described in Example 4, below.

Briefly, in the chorioallantoic assay, the potentially anti-angiogenic compound of interest is added to type I collagen pellets (Vitrogen), along with an angiogenic growth factor, such as bFGF. The samples are mixed and placed onto nylon meshes, and allowed to polymerize. After polymerization is complete, the meshes are placed onto the chorioallantoic membrane of 12 day old chick embryos and placed at 37'C for 24 hours. The embryos then injected with a fluorescent agent, such as FITC-dextran, and the meshes are fixed and mounted for observation under a fluorescent microscope.

. In the cornea pocket assay, hydron pellets containing the compound of interest and an angiogenic growth factor, such as bFGF, are implanted I to 2mm from the limbus of the cornea of rats or mice. Response is examined after a period of time, for example 5 days. The extent of angiogenesis is evaluated by measuring the capillaries migrating from the limb of the cornea.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 95%, 96%, 97%, 98%, or 99% identical to a nucleic acid sequence of the deposited cDNAs or a nucleic acid sequence shown in SEQ ID NO: I or SEQ ID NO: 3 will encode a polypeptide "having METH I or METH2 protein activity." In fact, since degenerate variants of these nucleotide sequences all encode the same polypeptide, this will be clear to the skilled artisan 5 even without

performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having N/IETH I or METH2 protein activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid).

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie, J. U. el al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306- 1310 (1990), whereinthe authorsindicatethatproteins are surprisingly tolerant of amino acid substitutions.

Vectors and Host Cells The present invention also relates to vectors which include the isolated DNA molecules of the present invention, host cells which are genetically engineered with the recombinant vectors, and the production of NffiTHI or NffiTH2 polypeptides or fragments thereof by recombinant techniques.

The polynucleotides may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The DNA insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few.

Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs will preferably include a translation initiating at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in E coli and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells; fungal cells, such as yeast cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH I 6a, pNH I 8A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia.

Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT I and pSG available from Stratagene- and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

In addition to the use of expression vectors in the practice of the present invention, the present invention further includes novel expression vectors comprising operator and promoter elements operatively linked to nucleotide sequences encoding a protein of interest. One example of such a vector is pHE4- 5 which is described in detail below.

As summarized in Figures 8 and 9, components of the pHE4-5 vector (SEQ ID NO: 12) include: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, 6) the lactose operon repressor gene (lac1q). The origin of replication (oriC) is derived from pUC 1 9 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences were made synthetically. Synthetic production of nucleic acid sequences is well known in the art. CLONTECH 95/96 Catalog, pages 215-216, CLONTECH, 1020 East Meadow Circle, Palo Alto, CA 94303. A nucleotide sequence encoding N4ETH1 (SEQ ID NO:2) or METH2 (SEQ ID NOA), is operatively linked to the promoter and operator by inserting the nucleotide sequence between the Ndel and Asp718 sites of the pHE4-5 vector.

As noted above, the pHE4-5 vector contains a laclq gene. Laclq is an allele of the lacl gene which confers tight regulation of the lac operator. Amann, E. et aL, Gene 69:301-315 (1988)- Stark, M., Gene 51:255-267 (1987). The laclq gene encodes a repressor protein which binds to lac operator sequences and blocks transcription of down-stream (i. e., T) sequences. However, the lae1q gene product dissociates from the lac operator in the presence of either lactose or certain lactose analogs, e.g., isopropyl B-D-thiogalactopyranoside (IPTG).

METH I or METH2 thus is not produced in appreciable quantities in uninduced host cells containing the pHE4-5 vector. Induction of these host cells by the addition of an agent such as IPTG, however, results in the expression of the METHI or METH2 coding sequence.

The promoter/operator sequences of the pHE4-5 vector (SEQ ID NO: 13) comprise a T5 phage promoter and two lac operator sequences. One operator is located 5' to the transcriptional start site and the other is located 3' to the same 0 site. These operators, when present in combination with the /aclq gene product, confer tight repression of down-stream sequences in the absence of a lac operon inducer, e.g., IPTG. Expression of operatively linked sequences located down-stream from the lac operators may be induced by the addition of a lac operon inducer, such as IPTG. Binding of a lac inducer to the laclq proteins results in their release from the lac operator sequences and the initiation of transcription of operatively linked sequences. Lac operon regulation of gene expression is reviewed in Devlin, T., TEXTBOOK OF BiomEmimy WITH CLINICAL CORRELATIONS, 4th Edition (I 997), pages 802 The pHE4 series of vectors contain all of the components of the pHE4-5 vector except for the METH I or METH2 coding sequence. Features of the pBE4 vectors include optimized synthetic T5 phage promoter, lac operator, and Shine-Delgarno sequences. Further, these sequences are also optimally spaced so that expression of an inserted gene may be tightly regulated and high level of expression occurs upon induction.

Among known bacterial promoters suitable for use in the production of proteins of the present invention include the E. coli lacl and 1acZ promoters, the T3 and T7 promoters, the gpt promoter, the lambda PR and PL promoters and the trp promoter. Suitable eukaryotic promoters include the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous Sarcoma Virus (RSV), and metallothionein promoters, such as the mouse metallothionein-I promoter.

The pRE4-5 vector also contains a Shine-Delgarno sequence 5' to the AUG initiation codon. Shine-Delgarno sequences are short sequences generally located about IO nucleotides up-stream (i.e., 5') from the AUG initiation codon.

These sequences essentially direct prokaryotic ribosomes to the AUG initiation codon.

Thus, the present invention is also directed to expression vector useful for the production of the proteins of the present invention. This aspect of the invention is exemplified by the p1-IE4-5 vector (SEQ ID NO: 12).

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (I 986).

The polypeptide may be expressed in a modified form, such as a fusion protein, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art. A preferred fusion protein comprises a heterologous region from immunoglobulin that is useful to solubilize proteins. For example, EP-A-0 464 53 3 (Canadian counterpart 2045 869) discloses fusion proteins comprising various portions of constant region of immunoglobin molecules together with another human protein or part thereof In many cases, the Fc part in a fusion protein is thoroughly advantageous for use in therapy and diagnosis and thus results, for example, in improved pharmacokinetic properties (EP-A 0232 262). On the other hand, for some uses it would be desirable to be able to

delete the Fe part after the fusion protein has been expressed, detected and purified in the advantageous manner described. This is the case when the Fe portion proves to be a hindrance to use in therapy and diagnosis, for example when the fusion protein is to be used as an antigen for immunizations. In drug discovery, for example, human proteins, such as the hIL5-receptor, have been fused with Fe portions for the purpose of high-throughput screening assays to identify antagonists of hIL See, D.

0 Bennett et al., J Mol. Recognition 8:52-58 (1995) and K. Johanson et al., J of Biol. Chem. 270(16):9459-9471 (1995).

The METHI or METH2 protein can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("FTLC") is employed for purification. Polypeptides of the present invention include naturally purified products, products of chemical synthetic procedures, and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionline residue, in some cases as a result of host-mediated processes.

METHI and METH2 Polypeptides and Fragments The invention further provides an isolated METH I polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in SEQ ID NO:2, or a peptide or polypeptide comprising a portion of the above polypeptides. The invention also provides an isolated METH2 polypeptide having the amino acid sequence encoded by the deposited cDNA, or the amino acid sequence in SEQ ID NOA, or a peptide or polypeptide comprising a portion of the above polypeptides.

METHI or METH2 polypeptides can be composed of amino acidsjoined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The METHI or METH2 polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and 1 5 in more detailed monographs, as well as in a voluminous research literature.

Modifications can occur anywhere in the NUETHI or METH2 polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given METHI or NIIETH2 polypeptide. Also, a given METH I or METH2 polypeptide may contain many types of modifications. METH I or MIETH2 polypeptides may be branched , for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic METHI or NIETH2 polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP- ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic to Press, New York, pgs, 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (I 990); Rattan et al., Ann NYAcad Sci 663:48-62 (1992).) It will be recognized in the art that some amino acid sequences of the METH I and METH2 polypeptides can be varied without significant effect of the structure or function of the protein. If such differences in sequence are contemplated, it should be remembered that there will be critical areas on the protein which determine activity.

The present inventors have shown that METHI and METH2 inhibit angiogenesis in vitro and in vivo. METHI and METH2 each contain a metalloprotease domain, a disintegrin domain, and TSP-tike domains. The metalloprotease

domain may be catalytically active. The disintegrin domain may play a role in inhibiting angiogenesis by interacting with integrins, since integrins are essential for the mediation of both proliferative and migratory signals. The present inventors have shown that peptides derived from the T SP-like domains of METHI and METH2 inhibit angiogenesis in vitro and in vivo.

Thus, the invention further includes variations of the METH I polypeptide which show substantial METH I polypeptide activity or which include regions of METHI protein such as the protein portions discussed below- and variations of the METH2 polypeptide which show substantial METH2 polypeptide activity or which include regions of METH2 protein such as the protein portions discussed below. Such mutants include deletions, insertions, inversions, repeats, and type substitutions. As indicated above, guidance concerning which amino acid changes are likely to be phenotypically silent can be found in Bowie, J.U., el aL, "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990).

Thus, the fragment, derivative or analog of the polypeptide of SEQ ID NO:2 or SEQ ID NO-4, or that encoded by the deposited cDNA, may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide, such as an IgG Fc fusion region peptide or 5 leader or secretory sequence or a sequence which is employed for purification of the mature polypeptide or a proprotein sequence. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

Of particular interest are substitutions of charged amino acids with another charged amino acid and with neutral or negatively charged amino acids. The latter results in proteins with reduced positive charge to improve the characteristics of the METHI or METH2 proteins. The prevention of aggregation is highly desirable. Aggregation of proteins not only results in a loss of activity but can also be problematic when preparing pharmaceutical formulations, because they can be immunogenic. (Pinckard et aL, Clin. Exp. Immunol. 2:331-340 (1967)- Robbins et aL, Diahetes 36:838-845 (1987); Cleland el aL, Cril. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993)).

As indicated, changes are preferably of a minor nature, such as conservative amino acid substitutions that do not significantly affect the folding or activity of the protein (see Table 3).

TABLE 3. Conservative Amino Acid Substitutions.

Aromatic Phenylalanine Tryptophan Tyrosine Hydrophobic Leucine Isoleucine Valine Polar Glutamine Asparagine Basic Arginine Lysine Histidine Acidic Aspartic Acid Glutamic Acid Small Alanine Serine Threonine Methionine Glycine Of course, the number of amino acid substitutions a skilled artisan would make depends on many factors, including those described above. Generally speaking, the number of amino acid substitutions for any given METHI or NMTH2 polypeptide will not be more than 50, 40, 30, 20, 10, 5, or 3.

Amino acids in the XIETH I and METH2 proteins of the present invention that are essential for function can be identified by methods known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and 1.5 Wells, Science 244:1081-1085 (1989)). The latter procedure introduces single alanine mutations at every residue in the molecule. The resulting mutant molecules are then tested for biological activity such as in vitro or in vivo inhibition of angiogenesis. Sites that are critical for inhibition of angiogenesis can also be determined by structural analysis such as crystallization, nuclear magnetic resonance or photoaffinity labeling (Smith etaL, J MoL BW 224:899-904 (1992) and de Vos et aL, Science 255:306-312 (1992)).

The polypeptides of the present invention are preferably provided in an isolated form. By "isolated polypeptide" is intended a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention.

Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host cell or from a native source. For example, a recombinantly produced version of the METHI or

WTH2 polypeptide can be substantially purified by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988).

The polypeptides ofthe present invention include the NIETH I polypeptide encoded by the deposited cDNA including the leader; the mature METHI polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein); a polypeptide comprising amino acids about I to about 950 in SEQ ID NO:2; a polypeptide comprising amino acids about 2 to about 950 in SEQ ID NO:2; a polypeptide comprising amino acids about 29 to about 950 in SEQ ID NO:2; a polypeptide comprising amino acids about 30 to about 950 in SEQ ID NO a polypeptide comprising the metalloprotease domain of METHI, amino acids 235 to 459 in SEQ ID NO:2; a polypeptide comprising the disintegrin domain of METHI, amino acids 460 to 544 in SEQ ID NO:2; a polypeptide comprising the first TSP-like domain of METHI, amino acids 545 to 598 in SEQ ID NO:2; a polypeptide comprising the second TSP-like domain of WM, amino acids 841 to 894 in SEQ ID NO:2- a polypeptide comprising the third TSP- like domain of METHI, amino acids 895 to 934 in SEQ ID NO:2,- a polypeptide comprising amino acids 536 to 613 in SEQ ID NO:2- a polypeptide comprising amino acids 549 to 563 in SEQ ID NO:2- the WTH2 polypeptide encoded by the deposited cDNA including the leader- the mature NMTH2 polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein); a polypeptide comprising amino acids about I to about 890 in SEQ ID NOA; a polypeptide comprising amino acids about 2 to about 890 in SEQ ID NOA,- a polypeptide comprising amino acids about 24 to about 890 in SEQ ID NOA; a 'de comprising amino acids about II 2 to about 890 in SEQ ID NOA a polypepti polypeptide comprising the metalloprotease domain of NIETH2, amino acids 214 to 439 in SEQ ID NOA; a polypeptide comprising the disintegrin domain of NIETH2, amino acids 440 to 529 in SEQ ID NOA; a polypeptide comprising the first TSP-11ke domain of WTH2, amino acids 530 to 583 in SEQ ID NOA; a polypeptide comprising the second TSP-like domain of NIIETH2, amino acids 837 to 890 in SEQ ID NOA; a polypeptide comprising amino acids 280 to 606 in SEQ to ID NOA; a polypeptide comprising amino acids 529 to 548 in SEQ ID NOA; as well as polypeptides which are at least 95% identical, and more preferably at least 96%, 97%, 98% or 99% identical to the polypeptides described above and also include portions of such polypeptides with at least 30 amino acids and more preferably at least 50 amino acids.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a reference amino acid sequence of a METHI or METH2 polypeptide is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each I 00 amino acids of the reference amino acid of the METHI or NtETH2 polypeptide. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence shown in SEQ ID NO:2 or SEQ ID NOA or to the amino acid sequence encoded by deposited cDNA clones can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 5371 1). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al., Comp. App. Biosci.

6:237-245 (1990). In a sequence alignment, the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty= 1, Joining Penalty=20, Randomization Group Length=O, Cutoff Score= 1, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the

length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global identity. For subject sequences truncated at the N- and C-termini, relative percenti to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total residues of the query sequence. Whether a residue is matched/aligned is determined by the results of the FASTDB sequence alignment.

This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are I 0 considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C-terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100residuequerysequencetodeterminepercen. Thedeletionoccursat the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a match/alignment of the first IO residues at the N-terminus. The I 0 unpaired residues represent I 0% of the sequence (number of residues at the N- and C-termini not matched/total number of residues in the query sequence) so I 0% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched, the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a I 00 residue query sequence. This time, the deletions are internal, so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case, the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are made for the purposes of the present invention.

The polypeptides of the present invention are useful as a molecular weight marker on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

In another aspect, the invention provides a peptide or polypeptide comprising an epitope-bearing portion of a polypeptide of the invention. The epitope of this polypeptide portion is an immunogenic or antigenic epitope of a polypeptide described herein. An "immunogenic epitope" is defined as a part of a protein that elicits an antibody response when the whole protein is the immunogen. On the other hand, a region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." The number of immunogenic epitopes of a protein generally is less than the number of antigenic epitopes. See, for instance, Geysen el al., Proc. Natl. Acad. Sci. USA 81:3 998- 4002 (1983).

As to the selection of peptides or polypeptides bearing an antigenic epitope (i.e., that contain a region of a protein molecule to which an antibody can bind), it is well known in that art that relatively short synthetic peptides that mimic part of a protein sequence are routinely capable of eliciting an antiserum that reacts with the partially mimicked protein. See, for instance, Sutcliffe, J. G. el, al., "Antibodiesthatreactwithpredetermined sites onproteins", Science 219:660-666 (1983). Peptides capable of eliciting protein-reactive sera are frequently represented in the primary sequence of a protein, can be characterized by a set of simple chemical rules, and are confined neither to immunodominant regions of intact proteins (i.e., immunogenic epitopes) nor to the amino or carboxyl terminals.

Antigenic epitope-bearing peptides and polypeptides of the invention are therefore useful to raise antibodies, including monoclonal antibodies, that bind specifically to a polypeptide of the invention. See, for instance, Wilson et al., Cell 37:767-778 (1984) at 777.

Antigenic epitope-bearing peptides and polypeptides of the invention preferably contain a sequence of at least seven, more preferably at least nine and most preferably between about at least about 15 to about 30 amino acids contained within the amino acid sequence of a polypeptide of the invention.

The epitope-bearing peptides and polypeptides of the invention may be produced by any conventional means. Houghten, R. A., "General method for the rapid solid-phase synthesis of large numbers of peptides: specificity of antigen-antibody interaction at the level of individual amino acids", Proe. Nad.

Acad. Sci. USA 82:5131-5135 (1985). This "Simultaneous Multiple Peptide Synthesis (SMPS)" process is further described in U.S. Patent No. 4,631,21 1 to Houghten el aL (I 986).

As one of skill in the art will appreciate, METH I or METH2 polypeptides of the present invention and the epitope-bearing fragments thereof described above can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. This has been shown, e.g., for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains ofthe constant regions ofthe heavy or light chains of mammalian immunoglobulins (EPA 394,827; Traunecker et al., Nature 331:84- 86 (1988)). Fusion proteins that have a disulfide-linked dimenic structure due to the IgG part can also be more efficient in binding and neutralizing other molecules than the monomeric METHI or METH2 protein or protein fragment alone (Fountoulakis et aL, J Biochem. 2 70:3 95 8-3 964 (1995)).

METHI and METH2 Polynucleotide and Polypeptide Fragments In the present invention, a "polynucleotide fragment" refers to a short polynucleotide having a nucleic acid sequence contained in the deposited clones or shown in SEQ ID NO: I or SEQ ID NO: 3. The short nucleotide fragments are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from the cDNA sequence contained in the deposited clones or the nucleotide sequence shown in SEQ ID NO: I or SEQ ID NO:3, These nucleotide fragments are useful as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., 50, 150, 500@ 600@ 2000 nucleotides) are preferred.

Moreover, representative examples ofMETH I or METH2 polynucleotide fragments include, for example, fragments having a sequence from about nucleotidenumber 1-50,51-100, 101-150, 151-200, 201-250,251-300,301-350, 351-400@401-450@ 451- 500,501-550,551-600,651-700I 701-7505 751-800,800- 8505 851-900@ 901-950@ 951-1000@ 1001-1050@ 1051-1100, 1101-1150, 1151- 1200, 1201-12507 1251-1300@ 1301- 1350@ 1351-1400, 1401-1450@ 1451-1500, 1501-1550, 1551-1600, 1601-1650@ 1651- 1700@ 1701-1750@ 1751-1800@ 1801-18507 1851-1900@ 1901-19505 1951-2000, or 2001 to the end of SEQ ID NO: I or SEQ ID N03 or the cDNA contained in the deposited clones. In this context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has biological activity. More preferably, these polynucleotides can be used as probes or primers as discussed herein.

In the present invention, a "polypeptide fragment" refers to a short amino acid sequence contained in SEQ ID NO:2 or SEQ ID NOA or encoded by the cDNA contained in the deposited clones. Protein fragments may be "free- standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragmentsfromaboutamino acidnumber 1-20,21-40,41-60, 61 100@ 102- 1207 121-140 @ 141-160@ 161-180@ 181-200,201-2205 221 260@ 261-280, or 281 to the end of the coding region or SEQ ID NO:2 or SEQ ID NOA Moreover, polypeptidefragmentscanbeabout2O,30,40,50, 60, 70, 80, 90, 100, 110, 120@ 130 @ 140, or 150 amino acidsinlength. Inthis context "about" includes the particularly recited ranges, larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either extreme or at both extremes.

Preferred polypeptide fragments include the secreted METH I or NMTH2 protein as well as the mature form. Further preferred polypeptide fragments include the secreted METHI or METH2 protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted NfETH I or METI-12 polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-3 0, can 1 0 be deleted from the carboxy terminus of the secreted METHI or XffiTH2 protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotide fragments encoding these METHI or METH2 polypeptide fragments are also preferred.

Particularly, N-terminal deletions of the METHI polypeptide can be described by the general formula m-950, where in is an integer from 2 to 949, where in corresponds to the position of the amino acid residue identified in SEQ ID NO:2. Preferably, N-terminal deletions of the NMTHI polypeptide of the invention shown as SEQ ID NO:2 include polypeptides comprising the amino acid sequence of residues: G-2 to S-950; N-3 to S-950; A-4 to S E-5 to S-950; R-6 to S-950; A-7 to S-950; P-8 to S G-9 to S S-10 to S-950; R-1 I to S-950; S- 1 2 to S-950; F- 1 3 to S G- 1 4 to S-950; P- 1 5 to S V- 1 6 to S- 950- P- 1 7 to S-950; T- 1 8 to S L- 1 9 to S L-20 to S-950; L-21 to S L-22 to S-950; A-23 to S-950; A-24 to S A-25 to S-950; L-26 to S-950; L- 27 to S-950; A-28 to S-950; V-29 to S-950; S-30 to S-950; D-31 to S-950; A-32 to S-950; L-33 to S G-34 to S-950; R-35 to S P-36 to S-950; S-37 to S-950; E-38 to S-950; E-39 to S-950; D-40 to S-950; E-41 to S-950; E-42 to S-950; L-43 to S-950; V-44 to S-950; V-45 to S-950; P-46 to S E-47 to S- 950; L-48 to S-950; E-49 to S R-50 to S A-51 to S P-52 to S-950; G-53 to S-950; H-54 to S-950; G-55 to S-950; T-56 to S-950; T-57 to S- 950; R-58 to S-950; L-59 to S-950; R-60 to S-950; L-61 to S-950; H-62 to S- 950- A-63 to S F-64 to S D-65 to S-950; Q-66 to S-950; Q-67 to S- 950; L-68 to S D-69 to S L-70 to S-950; E-71 to S-950; L-72 to S- 950- R-73 to S-950; P-74 to S D-75 to S-950; S-76 to S-950; S-77 to S F-78 to S L-79 to S-950; A-80 to S-950; P-81 to S-950; G-82 to S-950; F- 83 to S T-84 to S-950; L-85 to S-950; Q-86 to S-950; N-87 to S-950; V-88 to S-950; G-89 to S-950; R-90 to S K-91 to S-950; S-92 to S-950; G-93 to S-950; S-94 to S-950; E-95 to S-950; T-96 to S-950; P-97 to S-950; L-98 to S- 950; P-99 to S E-100 to S T-101 to S-950; D-102 to S-950; L-103 to S A-104to S-950; H-105 to S-950; C-106 to S F-107 to S-950; Y-108 to S S-109 to S G-1 10 to S-950; T-1 I I to S-950; V-1 12 to S-950; N- 113 to S-950; G-1 14 to S D-1 15 to S P-1 16 to S S-1 17 to S S-1 18 to S A-1 19 to S-950; A-120 to S-950; A-121 to S-950; L-122 to S- 950- S-123 to S-950; L-124 to S-950; C-125 to S-950; E-126 to S-950; G-127 to S-950; V-128 to S-950; R-129 to S-950; G-130 to S A-131 to S-950; F- 132 to S-950; Y-133 to S-950; L-134 to S L-135 to S G-136 to S E-137 to S-950; A-138 to S-950; Y-139 to S F-140 to S-950; 1-141 to S- 950; Q-142 to S-950; P-143 to S-950; L-144 to S-950; P-145 to S-950; A-146 to S-950; A-147 to S S-148 to S-950; E-149 to S-950; R-150 to S L- 151 to S A-152to S T-153 to S A-154 to S A-155 to S-950; P-156 to S-950; G-157 to S-950; E-158 to S K-159 to S-950; P-160 to S- 950- P-161 to S-950; A-162 to S-950; P-163 to S-950; L-164 to S-950; Q-165 to S-950; F-166 to S-950; H-167 to S-950; L-168 to S-950; L-169 to S-950; R- 170 to S R-171 to S-950;N-172to S R-173 to S Q-174 to S G- 1 75 to S-950; D- 1 76 to S-950; V- 1 77 to S-950; G- 1 78 to S-950; G- 1 79 to S- 950; T-180 to S-950; C-181 to S-950; G-182 to S V-183 to S-950; V-184 to S D-185 to S D-186 to S E-187 to S-950, P-188 to S R- 189 to S P-190 to S T-191 to S-950; G-192 to S-950; K-193 to S-950; A-194 to S-950; E-195 to S-950; T-196 to S E-197 to S-950; D-198 to S- 950; E-199 to S-950; D-200 to S-950; E-201 to S-950; G-202 to S-950; T-203 to S-950; E-204 to S-950; G-205 to S-950; E-206 to S-950; D-207 to S-950; E- 208 to S G-209 to S P-21 0 to S-950; Q-21 I to S-950; W-212 to S-950; S-213 to S P-214 to S-950; Q-215 to S D-216 to S P-217 to S-950- A-218 to S L-219 to S-950; Q-220 to S-950; G-221 to S-950; V-222 to S G-223 to S-950; Q-224 to S-950; P-225 to S-950; T-226 to S-950; G- 227 to S-950; T-228 to S-950; G-229 to S-950; S-230 to S-950; 1-231 to S-950; R-232 to S-950; K-233 to S-950; K-234 to S-950; R-235 to S-950; F-236 to S- 950; V-237 to S-950; S-238 to S S-239 to S H-240 to S-950; R-241 to S Y-242 to S-950; V-243 to S-950; E-244 to S-950; T-245 to S-950; M- 246 to S-950; L-247 to S-950; V-248 to S A-249 to S-950; D-250 to S Q-251 to S-950; S-252 to S-950; M-253 to S-950; A-254 to S E-255 to S- 950; F-256 to S-950; H-257 to S-950; G-258 to S S-259 to S G-260 to S-950; L-261 to S-950; K-262 to S-950; H-263 to S-950; Y-264 to S-950; L- 265 to S-950; L-266 to S T-267 to S L-268 to S-950; F-269 to S S-270 to S-950; V-271 to S-950; A-272 to S-950; A-273 to S R-274 to S- 950. L-275 to S Y-276 to S K-277 to S H-278 to S P-279 to S-950; S-280 to S-950; 1-281 to S-950; R-282 to S-950; N-283 to S-950; S- 284 to S-950; V-285 to S-950; S-286 to S-950; L-287 to S V-288

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to S N-833 to S A-834 to S-950; 1-835 to S P-836 to S-950; T-837 to S-950; F-838 to S-950; S-839 to S-950; A-840 to S-950; W-841 to S- 950; V-842 to S-950; 1-843 to S-950; E-844 to S-950; E-845 to S W-846 to S-950; G-847 to S-950; E-848 to S C-849 to S-950; S-850 to S K- 851 to-S-950; S-852 to S-950; C-853 to S-950; E-854 to S-950; L-855 to S-950; G-856 to S-950; W-857 to S-950; Q-858 to S R-859 to S R-860 to S- 950; L-861 to S-950; V-862 to S-950; E-863 to S C-864 to S R-865 to S-950; D-866 to S-950; 1-867 to S-950; N-868 to S-950; G-869 to S-950; Q-870 to S P-871 to S-950; A-872 to S-950: S-873 to S-950; E-874 to S C-875 to S-950; A-876 to S K-877 to S-950; E-878 to S-950; V-879 to S- 950; K-880 to S-950; P-881 to S-950; A-882 to S-950; S-883 to S-950; T-884 to S-950; R-885 to S-950; P-886 to S-950; C-887 to S A-888 to S D- 889 to S-950; H-890 to S-950; P-891 to S C-892 to S P-893 to S-950; Q-894 to S W-895 to S Q-896 to S L-897 to S G-898 to S- 950- E-899 to S-950; W-900 to S-950; S-901 to S-950; S-902 to S-950; C-903 to S-950; S-904 to S K-905 to S T-906 to S-950; C-907 to S G- 908 to S K-909 to S-950; G-91 0 to S-950; Y-911 to S-950; K-912 to S-950; K-913 to S-950; R-914 to S-950; S-915 to S-950; L-916 to S-950; K-917 to S- 950; C-918 to S-950; L-919 to S-950; S-920 to S-950; H-921 to S-950; D-922 to S-950; G-923 to S G-924 to S-950; V-925 to S L-926 to S-950; S- 927 to S-950; H-928 to S , E-929 to S-950; S-930 to S-950,- C-931 to S-950; D-932 to S-950; P-933 to S-950; L-934 to S-950, K-935 to S-950; K-936 to S- 950; P-937 to S-950; K-938 to S H-939 to S F-940 to S-950; 1-941 to S-950; D-942 to S-950; F-943 to S-950; C-944 to S T-945 to S-950; of SEQ ID NO: 2.

Moreover, C-terminal deletions of the METHI polypeptide can also be described by the general formula I -n, where n is an integer from 2 to 95 0, where n corresponds to the position of amino acid residue identified in SEQ ID NO:2.

Preferably, C-terminal deletions of the METHI polypeptide of the invention shown as SEQ ID NO: 2 include polypeptides comprising the amino acid sequence of residues: M-1 to C-949; M-1 to E M-1 to A M-1 to M M-1 to T M-1 to C-944; M-1 to F-943; M-1 to D M-1 to 1-941; M-1 to F-940; M-1 to H-939; M-1 to K M-1 to P M-1 to K-936; M-1 to K-935; M-1 to L-934; M-1 to P-933; M-1 to D-932; M-1 to C-93 I M-1 to S-930; M-1 to E- 929; M-1 to H-928; M-1 to S-927; M-1 to L-926; M-1 to V M-1 to G M-1 to G-923; M-1 to D-922; M-1 to H-921; M-1 to S-920; M-1 to L M-1 to C-918; M- I to K-917 M- I to L-916; M- I to S-915; M- I to R-914; M- I to K- 913; M- I to K-912; M- I to Y-91 1; M- I to G-91 0; M- I to K-909 - M- I to G-908 M- I to C M- I to T-906; M- I to K-905 M- I to S M- I to C-903 M-I to S M- I to S-901 M- I to W M- I to E M- I to G-898 M- I to L- 897; M- I to Q M- I to W-895; M- I to Q-894; M- I to P-893; M- I to C-892; M-1 to P-891; M-1 to H-890; M-1 to D-889; M-1 to A-888; M-1 to C-887; M-1 to P-886; M-I to R-885; M-1 to T-884; M-1 to S-883; M-1 to A-882; M-1 to P- 881; M-1 to K M-1 to V-879; M-1 to E-878; M-1 to K M-1 to A-876; M-1 to C-875; M-I to E M-1 to S M-1 to A M-1 to P-871; M-1 to Q M-1 to G-869; M-1 to N-868; M-1 to 1-867; M-1 to D M-1 to R- 865; M- I to C M- I to E-863; M- I to V-862; M- I to L-861 M- I to R-860; M-1 to R-859; M-1 to Q-858; M-1 to W-857; M-1 to G M-1 to L M-1 to E-854; M-1 to C-853; M-1 to S-852; M-1 to K-85 1; M-1 to S-850; M-1 to C- 849; M- I to E-848; M- I to G-847; M- I to W-846; M- I to E-845; M- I to E-844; M-1 to 1-843; M-1 to V-842; M-1 to W-841; M-1 to A M-1 to S-839; M-1 to F-838; M-1 to T-837; M-1 to P-836; M-1 to 1-835; M-1 to A-834; M-1 to N- 833- M-1 to F M-1 to S-83 I- M-1 to E-830; M-1 to K-829; M-1 to K M- I to K M- I to K-826; M-I to V-825; M- I to F-824; M- I to Y-823; M- I to T M- I to Y-82 1; M- I to K-820; M- I to 1-819; M- I to K-818 M- I to P- 817; M- I to R-816 M- I to L-815; M- I to A-814 - M- I to N-813; M- I to G-812, M- I to V-81 1; M- I to T-8 I 0-M- I to L-809; M- I to V-808; M- I to Q-807; M- I to 1-806; M-1 to T-805; M-1 to L M-1 to P-803; M-1 to E M-1 to K-801; M-1 to L M-1 to P M-1 to S-798; M-1 to F M-1 to S-796; M-1 to R-795; M- I to 1-794; M- I to R-793; M- I to E-792; M- I to L-79 1; M- I to A-790; M- I to A M- I to S-788; M- I to S-787; M- I to G-786; M- I to S- 785; M- I to Y-784; M- I to R-783; M- I to L-782; M- I to V-78 1; M- 1 to V-780; M-1 to G M-1 to K-778; M-1 to Y-777; M-1 to M-776; M-1 to 1-775; M-1 to D M-1 to Q-773; M-1 to E-772; M-1 to L M-1 to T M-1 to S- 769; M- I to L-768 M-I to T-767; M- I to Y-766; M- I to D-765; M- I to G M- I to N-763 M- I to L M-I to 1-761 M- I to Y-760; M- I to T-759; M-I to G-758; M-1 to D-757; M-1 to A M-1 to A-755; MA to K-754; M-1 to 1-753; M-1 to A-752; M-1 to L M-1 to F-750; M-1 to S-749; M-1 to G-748; M-1 to N M-1 to N M-1 to R M-1 to S M-1 to G M-1 to R-742; M-1 to Q-741; M-1 to N M-1 to R-739; M-1 to Q MA to K M-1 to V-736; M-1 to E-735; M-1 to 1 M-1 to N M-1 to T- 732- M-1 to A M-1 to G M-1 to T-729; M-1 to P-728; M-1 to 1 M-1 to T-726; M-1 to 1-725; M-1 to 1-724; M-1 to D-723; M-1 to H-722; M-1 to Y-72 1; M- I to G-720; M- I to P-719; M- I to K-718; M- I to A-717; M- I to S- 716; M- I to T-715 M-I to V M- I to S-713 M- I to G M- I to S-71 1; M-1 to 1-710; M-1 to K M-1 to K-708; M-1 to C-707; M-1 to T-706; M-1 to S M-1 to G M-1 to N M-1 to G-702; M-1 to G M-1 to C- 700; M- I to V M- I to G-698; M- I to C-697; M- I to K-696; M- I to D-695; M- I to F-694; M- I to K-693; M- I to K-692; M- I to K-69 1; M- I to S-690; M- I to D M-1 to 1-688; M-1 to 1- 687; M-1 to R-686; M-1 to D-685; M-1 to C- 684; M- I to G-683; M- I to A M- I to K-68 1; M- I to V-680; M- I to C M- I to Q-678; M- I to G-677, M- I to Q-676; M- I to V-675 M- I to C-674; M- I to V-673 M- I to S M- I to T-671 M- I to S-670; M- I to D M- I to P- 668; M- I to S-667; M- I to C-666; M- I to P-665; M- I to T-664; M- I to G-663; M- I to D-662; M- I to V-66 1; M- I to V-660; M-I to K-65 9; M- I to P-65 8 M- I to Q M- I to L-656; M- I to V-65 5 M- I to F M- I to F-653; M- I to Y- 652; M-1 to G-651; M-1 to 1-650; M-1 to G-649; M-1 to K M-1 to A M-1

to Q M-1 to C M-1 to 1 M-1 to L M-1 to K-642; M-1 to C M-1 to R M-1 to D-639; M-1 to K-638; M-1 to P-637; M-1 to S- 636; M-1 to V-635; M-1 to G-634; M-1 to A-633; M-1 to Y-632; M-1 to K-631; M- I to P-63 0; M- I to 1-629; M- I to W-628; M- I to E-627; M- I to V-626; M- I to A-625; M- I to P-624; M- I to G-623; M- I to S-622; M- I to G-62 1; M- I to F- 620; M-1 to S-619; M-1 to A-618; M-1 to K-617; M-1 to S M-1 to F-615; M- I to E-614; M- I to N-613; M- I to H-612; M- I to A-61 1; M- I to E-6 I 0; M- I to C-609; M-1 to Q-608; M-1 to E-607; M-1 to E M-1 to R-605; M-1 to F- 604; M-1 to T M-1 to K M-1 to G-601; M-1 to N-600; M-1 to N-599; M-1 to D-598; M-1 to P-597; M-1 to C-596; M-1 to D-595; M-1 to E-594; M-1 to L-593; M-1 to N-592; M-1 to C-591; M-1 to S-590; M-1 to R-589; M-1 to Y-588; M-1 to R-587; M-1 to V-586; M-1 to R-585; M-1 to K-584; M-1 to G-583)- M-1 to E-582; M-1 to C-581; M-1 to Y-580; M-1 to K-579; M-1 to G-578; M-1 to G M-1 to N-576; M-1 to K M-1 to P-574; M-1 to V M-1 to P- 572; M-1 to N-571; M-1 to D M-1 to C-569; M-1 to E-568; M-1 to R M-1 to M-566; M-1 to T-565; M-1 to Y-564; M-1 to Q-563; M-1 to V-562; M-1 to G-561; M-1 to G-560; M-1 to G-559; M-1 to C-558; M-1 to T-557; M-1 to R- 556; M-1 to S-555; M-1 to C M-1 to D M-1 to G M-1 to W-551; M-1 to P-550; M-1 to G-549; M-1 to W-548; M-1 to M-547; M-1 to G-546; M-1 to W-545; M-1 to S-544; M-1 to G-543; M-1 to H M-1 to F M-1 to P-540; M-1 to T-539; M-1 to D-538; M-1 to F-537; M-1 to H-536; M-1 to K- 535- M-1 to R M-1 to D-533; M-1 to T-532; M-1 to K-531; M-1 to N-530; M- I to V-529; M- I to C-528 @- M- I to K - M- I to G-526; M- I to N-525; M- I to 1-524; M- I to C-523 M- I to W-522; M- I to K-52 1; M- I to G-520; M- I to E- 519; M-1 to G-518; M-1 to C-517; M-1 to S-516; M-1 to T-515; M-1 to G-514,- M-1 to D-513; M-1 to A M-1 to W-51 1; M-1 to P-510; M-1 to F-509; M-1 to H M-1 to K-507; M-1 to T M-1 to Q M-1 to C M-1 to V- 503- M-1 to L M-1 to V-501; M-1 to G-500; M-1 to G-499; M-1 to S-498; M- I to T-497; M- I to G-496; M-I to T-495 M- I to C M- I to W-493; M- I to L M-1 to T-491; M-1 to S-490; M-1 to C M-1 to T-488; MA to S- 487; M-1 to A-486; M-1 to A-485; M-1 to D M-1 to P M-1 to C-482; M- I to H-48 1; M- I to K-480; M- I to S-479; M- I to D-478; M- I to E-477; M- I to G M- I to F-475; M- I to T-474; M- I to F-473; M- I to Q-472; M- I to C- 47 1; M- I to Q M- I to R M- I to N-468; M- I to A-467; M- I to D-466; M- I to Y-465; M- I to S-464; M- I to T-463; M- I to G-462; M- I to P-46 1; M- I to L-460; M-1 to D M-1 to G-458; M-1 to P-457; M-1 to L MA to Q- 455; M-1 to 1-454; M-1 to P-453; M-1 to N-452; M-1 to Q-451; M-1 to P-450; M-1 toK-449; M-1 toD-448; M-1 to M-447; M-1 to L M-1 to C-445; M-1 to E-444; M- I to G-443 M- I to H-442; M- I to G-441 M- I to N-440 M- I to D-43 9; M- I to L-43 8; M- I to F-43 7; M- I to S-43 6; M- I to T-43 5; M- I to 1-434; M- I to M-43 3; M- I to Y-43 2; M- I to A-43 1; M- I to S-43 0; M- I to C-429; M- 1 to P-428; M- I to S-427; M- I to W-426; M- I to P-425 M- I to Q M- I to S- 423; M- I to H-422; M- I to D-42 1; M- I to L-420; M- I to N-419 M- I to S-418; M- I to L-417; M- I to M-416; M- I to S-415; M- I to A-414; M- I to M-413; M- I to M-412; M- I to H-41 1; M- I to S-41 0- M- I to D-409; M- I to Q-408; M- I to N M-1 to V-406; M-1 to G M-1 to N M-1 to L-403; M-1 to S- 402; M-1 to A M-1 to C M-1 to Q-399; MA to K M-1 to A M- I to D-3 96; M- I to D M-1 to H-394; M-1 to P M-1 to M M-1 to N-391; MA to F-390; M-1 to V-389; M-1 to H M-1 to G-387; M-1 to L- 386; M-1 to E M-1 to H-384; M-1 to A M-1 to T-382, M-1 to T M-1 to F-380; M-1 to A-379; M-1 to A-378; M-1 to Q-377; M-1 to L M-1 to G-375; M-1 to D-374; M-1 to D M-1 to E-372; M-1 to 1-371; M-1 to V- 3 70; M- I to S-3 69; M- I to C-3 68; M- I to S-3 67; M- I to R-3 66)- M- I to S-3 65; M- I to P-3 64; M- I to D-3 63; M- I to C-3 62- M- I to V-3 61 M- I to T-3 60; M- I to G-359; M-1 to V-358; M-1 to D-357; M-1 to A M-1 to M-355; MA to G-354; MA to L-353; MA to T-352; MA to D MA to C-350; MA to T- 349; MA to Q MA to S MA to G-346; MA to C-345; MA to L M- I to D-3 43; M- I to Q-3 42; M- I to R-3 4 1; M- I to T-3 40; M- I to F-3 3 9; M- I to L-338; MA to 1 MA to A MA to T-335; MA to D-334; MA to Y- 333; MA to H-332; MA to E-33 1; MA to A M-1 to D MA to R MA to D-327; MA to S-326; M-1 to P-325; MA to P-324; M-1 to N-323; M-1 to H M-1 to Q-321 M-1 to K-320; M-1 to Q-319; M-1 to W-318; M-1 to N M-1 to C M-1 to F M-1 to N M-1 to R-313; M-1 to L- 312- M-1 to T-31 1; M-1 to L-3 10; M-1 to A M-1 to A-308; M-1 to N M-1 to S-306; M-1 to T-305; M-1 to V-304; M-1 to E-303; M-1 to P-302; M-1 to G-301; M-1 to K-300; M-1 to Q-299; M-1 to E-298; M-1 to D-297; MA to H M-1 to 1 M-1 to V-294; M-1 to L M-1 to 1-292; M-1 to K M- I to V-290; M- I to V-289; M- I to V M- I to L-287; M- I to S M- I to V M-1 to S M-1 to N M-1 to R-282; M-1 to 1-281; M-1 to S- 280; M- I to P-279; M- I to H-278; M- I to K-277; M- I to Y-276; M- I to L-275; M-1 to R-274; M-1 to A-273; M-1 to A M-1 to V-271; M-1 to S-270; M-1 to F M-1 to L M-1 to T M-1 to L M-1 to L-265; M-1 to Y- 264; M-1 to H-263; M-1 to K M-1 to L-261; M-1 to G-260; M-1 to S-259; M-1 to G MA to H-257; M-1 to F-256; M-1 to E-255; M-1 to A-254; M-1 to M-253; M- I to S M- I to Q-25 1; M- I to D-250; M- I to A M- I to V-248; M-1 to L M-1 to M-246; M-1 to T-245; M-1 to E M-1 to V- 243; M-1 to Y-242; M-1 to R-241; M-1 to H-240; M-1 to S-239; M-1 to S-238; M-1 to V-237; M-1 to F M-1 to R-235; M-1 to K-234; M-1 to K-233; M-1 to R-232; M-1 to 1-23 1; M-1 to S M-1 to G-229; M-1 to T-228; M-1 to G- 227; M-1 to T-226; M-1 to P-225; M-1 to Q-224; M-1 to G-223; M-1 to V-222,- M- I to G-22 1; M- I to Q M- I to L-219; M- I to A-218; M- I to P-217; M- I to D M-1 to Q-215; M-1 to P M-1 to S-213; MA to W-212; M-1 to Q-21 I - M- I to P-21 0M- I to G-209; M- I to E-208 M- I to D-207; M- I to E- 206- M- I to G-205; M- I to E M- I to T-203; M- I to G-202; M- I to E-20 1; M-1 to D-200; M-1 to E-199; M-1 to D-198; M-1 to E M-1 to T-196; M-1 to E-195; M-1 to A M-1 to K-193; M-1 to G-192; M-1 to T-191; M-1 to P- 190; M-1 to R M-1 to P-188; M-1 to E M-1 to D M-1 to D M-1 to V-184; M-1 to V M-1 to G-182; M-1 to C M-1 to T-180; M-1 to G-179; M-1 to G M-1 to V-177; M-1 to D M-1 to G-175; M-1 to Q-174;

M-1 to R M-1 to N M-1 to R-171; M-1 to R-170; M-1 to L- 169; M-1 to L-168, M-1 to H-167; M-1 to F M-1 to Q-165; M-1 to L-164; M-1 to P-163; M-1 to A M-1 to P M-1 to P-160; M-1 to K M-1 to E-158; M-1 to G-157; M-1 to P M-1 to A-155; M-1 to A-154; MA to T- 153- M-1 to A-152; M-1 to L-151; M-1 to R-150; M-1 to E-149; M-1 to S-148; M-1 to A-147; M-1 to A-146; M-1 to P M-1 to L-144; M-1 to P-143; M-1 to Q-142; M-1 to 1 M-1 to F-140; M-1 to Y-139; M-1 to A-138; M-1 to E- 137; M-1 to G-136; M-1 to L-135; M-1 to L-134; M-1 to Y M-1 to F-132; M-1 to A M-1 to G M-1 to R-129; MA to V-128; M-1 to G-127; M-1 to E-126; M-1 to C-125; M-1 to L-124; M-1 to S-123; M-1 to L-122; M-1 to A- 121- M-1 to A-120; M-1 to A-1 19- M-1 to S-1 18- M-1 to S-1 IT M-1 to P-1 16; M-1 to D-1 15; M-1 to G-1 14- M-1 toN-1 13; M-1 to V-1 12- M-1 to T-1 I 1; M-1 to G-1 10- M-1 to S M-1 to Y-108; M-1 to F M-1 to C-106; M-1 to H- 105; M-1 to A M-1 to L-103; M-1 to D-102; M-1 to T-101; M-1 to E M-1 to P-99; M-1 to L-98; M-1 to P-97; M-1 to T-96; M-1 to E-95; M-1 to S-94; M-1 to G-93; M-1 to S-92; M-1 to K-91 M-1 to R MA to G-89; M-1 to V- 88; M-1 to N-87; M-1 to Q-86; M-1 to L-85; M-1 to T-84; M-1 to F-83; MA to G-82; M-1 to P-81; M-1 to A M-1 to L-79; M-1 to F-78; M-1 to S-77; M-1 to S M-1 to D-75; M-1 to P-74; MA to R M-1 to L MA to E M- I to L-70; M-1 to D M-1 to L M-1 to Q-67; M-1 to Q-66; M-1 to D-65; M-1 to F-64; M-1 to A-63; M-1 to H-62; M-1 to L-61; M-1 to R M-1 to L- 59; M-1 to R M-1 to T M-1 to T M-1 to G M-1 to H M-1 to G-53 M- I to P-52; M- I to A-5 1; M- I to R-50; M- I to E-49; M- I to L-48; M- I to E-47; M- I to P-46; M- I to V-45; M- I to V-44; M- I to L-43; M- I to E-42; M- I to E-41; M-1 to D M-1 to E-39; M-1 to E-38; M-1 to S-37; M-1 to P-36; M-1 to R-35; M-1 to G M-1 to L-33; M-1 to A-32; M-1 to D-3 1; MA to S- 30- M-1 to V M-1 to A-28; M-1 to L-27; M-1 to L-26; M-1 to A-25; M-1 to A M-1 to A M-1 to L M-1 to L-21; M-1 to L-20; M-1 to L M-1 to T-18; M-1 to P-17; M-1 to V-16; M-1 to P-15; M-1 to G M-1 to F M- I to S-12; M-1 to R-1 1; M-1 to S M-1 to G-9; M-1 to P M-1 to A-7; of SEQ ID NO:2. For example, any of the above listed N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted METH I polypeptide.

Moreover, N-terminal deletions of the METH2 polypeptide can be described by the general formula m-890, where in is an integer from 2 to 889, where in corresponds to the position of the amino acid residue identified in SEQ ID NOA. Preferably, N-terminal deletions of the METI-12 polypeptide of the invention shown as SEQ ID NO: 4 include polypeptides comprising the amino acid sequence of residues: F-2 to L-890; P-3 to L-890; A-4 to L-890; P-5 to L-890; A-6 to L-890; A-7 to L P-8 to L R-9 to L-890; W-10 to L-890; LA I to L-890; P-12 to L-890; F-13 to L L-14 to L-890; L-15 to L L-16 to L-890; L- 1 7 to L-890; L- 1 8 to L L- 1 9 to L-890; L-20 to L-890; L-21 to L- 890; L-22 to L-890; P-23 to L-890; L-24 to L A-25 to L-890; R-26 to L- 890; G-27 to L A-28 to L-890; P-29 to L-890; A-30 to L-890; R-31 to L- 890; P-32 to L-890; A-33 to L-890; A-34 to L G-35 to L-890; G-36 to L- 890; Q-37 to L A-38 to L-890; S-39 to L-890; E-40 to L L-41 to L- 890- V-42 to L V-43 to L-890; P-44 to L-890; T-45 to L-890; R-46 to L- 890; L-47 to L P-48 to L G-49 to L S-50 to L A-51 to L- 890; G-52 to L-890; E-53 to L-890; L-54 to L-890; A-55 to L L-56 to L- 890; H-57 to L-890; L-58 to L-890; S-59 to L-890; A-60 to L-890; F-61 to L- 890; G-62 to L-890; K-63 to L G-64 to L-890; F-65 to L V-66 to L- 890; L-67 to L-890; R-68 to L L-69 to L-890; A-70 to L-890; P-71 to L- 890; D-72 to L-890; D-73 to L-890; S-74 to L F-75 to L L-76 to L- 890; A-77 to L P-78 to L E-79 to L F-80 to L K-81 to L- 890; 1-82 to L E-83 to L R-84 to L L-85 to L G-86 to L- 890; G-87 to L-890; S-88 to L-890; G-89 to L-890; R-90 to L A-91 to L- 890; T-92 to L-890; G-93 to L-890; G-94 to L-890; E-95 to L-890; R-96 to L- 890- G-97 to L-890; L-98 to L-890; R-99 to L-890; G-100 to L C-101 to L- 890- F-102 to L-890; F-103 to L S-104 to L-890; G-105 to L-890; T-106 to L-890; V-107 to L N-108 to L G-109 to L E-1 10 to L-890; P- I I I to L-890; E-1 12 to L S-1 13 to L-890; L-1 14 to L-890; A-1 15 to L-890; A- 1 16 to L-890; V- 1 17 to L-890; S- 1 18 to L-890; L- 1 19 to L-890; C- 1 20 to L- 890; R-121 to L-890; G-122 to L-890; L-123 to L-890; S-124 to L-890; G-125 to to L S-126 to L F-127 to L-890; L-128 to L-890; L-129 to L-890; D- 130 to L G-131 to L-890; E-132 to L-890; E-133 to L-890; F-134 to L-890; T-135 to L-890; 1-136 to L Q-137 to L-890; P-138 to L Q-139 to L- 890; G-140 to L A-141 to L-890; G-142 to L G-143 to L S-144 to L-890; L-145 to L-890; A-146 to L Q-147 to L-890; P-148 to L-890; H- 149 to L-890; R-150 to L-890; L-151 to L Q-152 to L-890; R-153 to L-890; W-154 to L-890; G-155 to L P-156 to L A-157 to L-890; G-158 to L- 890- A- 1 59 to L-890; R- 1 60 to L-890; P- 1 61 to L-890; L- 1 62 to L-890; P- 1 63 to L-890; R-1 64 to L G- 1 65 to L-890; P- 1 66 to L E- 1 67 to L-890; W- 168 to L-890; E- 1 69 to L-890; V- 1 70 to L-890; E- 1 71 to L-890; T- 1 72 to L-890; G-173 to L-890; E-174 to L G-175 to L-890; Q-176 to L-890; R-177 to L- 890; Q-178 to L E- 179 to L R- 1 80 to L G- 1 81 to L D- 1 82 to L-890; H- 1 83 to L-890; Q- 1 84 to L E- 1 85 to L D- 1 86 to L-890; S- 187 to L-890; E- 1 88 to L-890,- E- 1 89 to L-890; E- 1 90 to L S- 1 91 to L Q- 1 92 to L-890; E- 1 93 to L-890; E- 1 94 to L-890; E- 1 95 to L A- 1 96 to L- 890; E-197 to L G-198 to L-890; A-199 to L-890; S-200 to L-890; E-201 to L-890; P-202 to L P-203 to L-890; P-204 to L P-205 to L L- 206to L G-207 to L-890; A-208 toL T-209 to L-890; S-210toL-890; R-211 to L T-212 to L-890; K-213 to L-890; R-214 to L-890; F-215 to L- 890- V-216 to L S-217 to L E-218 to L A-219 to L-890; R-220 to L-890; F-221 to L-890; V-222 to L-890; E-223 to L-890; T-224 to L-890; L- 225 to L-890; L-226 to L-890; V-227 to L-890; A-228 to L-890; D-229 to L-890; A-230 to L-890; S-231 to L-890; M-232 to L-890,- A-233 to L-890,- A-234 to L- 890; F-235 to L-890; Y-236 to L-890; G-237 to L-890; A-238 to L-890; D-239 to L-890; L-240 to L-890; Q-241

to L-890; N-242 to L-890; H-243 to L-890; I-244 to L L-245 to L T-246 to L L-247 to L-890; M-248 to L S-249 to L-890; V-250 to L A-251 to L-890; A-252 to L R-253 to L- 890; 1-254 to L-890; Y-255 to L-890; K-256 to L-890; H-257 to L-890; P-258 to L S-259 to L-890: 1-260 to L K-261 to L-890; N-262 to L S- 263 to L-890; 1- 264 to L N-265 to L-890; L-266 to L-890; M-267 to L-890; V-268 to L V-269 to L K-270 to L V-271 to L-890; L-272 to L-890- 1-273 to L-890; V-274 to L-890; E-275 to L-890; D-276 to L-890; E-277 to L K-278 to L-890; W-279 to L G-280 to L P-281 to L-890; E- 282 to L V-283 to L-890; S-284 to L-890; D-285 to L-890; N-286 to L G-287 to L-890; G-288 to L L-289 to L T-290 to L-890; L-291 to L- 890; R-292 to L-890; N-293 to L F-294 to L-890; C-295 to L-890; N-296 to L-890; W-297 to L Q-298 to L-890; R-299 to L-890; R-300 to L-890; F- 301 to L-890; N-302 to L-890; Q-303 to L P-304 to L-890, S-305 to L D-306 to L-890; R-307 to L H-308 to L P-309 to L E-3 10 to L-890; H-311 to L-890; Y-312 to L D-313 to L-890; T-314 to L A-315 to L-890; 1- 316 to L-890; L-317 to L-890; L-318 to L-890; T-319 to L-890; R- 320 to L Q-321 to L N-322 to L-890; F-323 to L-890; C-324 to L G-325 to L Q-326 to L E-327 to L-890; G-328 to L L-329 to L- 890; C-330 to L-890; D-331 to L-890; T-332 to L L-333 to L-890; G-334 to L-890; V-335 to L-890; A-336 to L-890; D-337 to L-890; 1-338 to L-890;

G- 339 to L-890; T-340 to L-890; 1-341 to L C-342 to L-890; D-343 to L P-344 to L-890; N-345 to L-890; K-346 to L-890; S-347 to L-890; C-348 to L- 890; S-349 to L-890; V-350 to L-890; 1-351 to L-890; E-352 to L D-353 to L-890; E-354 to L-890; G-355 to L L-356 to L Q-357 to L-890; A- 358 to L-890; A-359 to L-890; H-360 to L T-361 to L L-362 to L A-363 to L-890; H-364 to L-890; E-365 to L-890; L-366 to L G-367 to L- 890; H-368 to L V-369 to L L-370 to L-890; S-371 to L-890; M-372 to L-890; P-373 to L-890; H-374 to L D-375 to L D-376 to L-890; S- 377 to L-890; K-378 to L P-379 to L-890; C-380 to L-890; T-381 to L R-382 to L L-383 to L F-384 to L G-385 to L-890; P-386 to L- 890; M-387 to L-890; G-388 to L-890; K-389 to L-890; H-390 to L-890; H-391 to L-890; V-392 to L-890; M-393 to L A-394 to L P-395 to L-890; L- 396 to L F-397 to L V-398 to L H-399 to L L-400 to L-890; N-401 to L-890; Q-402 to L-890; T-403 to L-890; L-404 to L P-405 to L- 890- W-406 to L-890; S-407 to L-890; P-408 to L-890; C-409 to L S-410 to L-890; A-411 to L-890; M-412 to L-890; Y-413 to L L-414 to L-890; T- 415 to L-890; E-416 to L L-417 to L L-418 to L D-419 to L G-420 to L-890; G-421 to L-890; H-422 to L-890; G-423 to L-890; D-424 to L- 890; C-425 to L-890; L-426 to L-890; L-427 to L D-428 to L-890; A-429 to L-890; P-430 to L-890; G-431 to L-890; A-432 to L-890; A-433 to L-890; L-434 to L-890; P-435 to L-890; L-436 to L-890; P-437 to L-890; T-438 to L-890, G-439 to L-890; L-440 to L-890; P-441 to L G-442 to L R-443 to L- 890; M-444 to L A-445 to L L-446 to L-890; Y-447 to L Q-448 to L-890; L-449 to L-890; D-450 to L-890; Q-451 to L-890; Q-452 to L C- 453 to L-:890; R-454 to L-890; Q-455 to L-890; 1-456 to L F-457 to L-890; G-458 to L-890; P-459 to L-890; D-460 to L-890; F-461 to L R-462 to L- 890- H-463 to L-890; C-464 to L-890; P-465 to L-890; N-466 to L-890; T-467 to L-890; S-468 to L-890; A-469 to L Q-470 to L D-471 to L V- 472 to L-890; C-473 to L-890; A-474 to L-890; Q-475 to L-890; L-476 to L-890, W-477 to L C-478 to L H-479 to L-890; T-480 to L D-481 to L- 890; G-482 to L-890; A-483 to L-890; E-484 to L P-485 to L-890; L-486 to L C-487 to L-890; H-488 to L T-489 to L-890; K-490 to L N- 491 to L-890; G-492 to L S-493 to L-890; L-494 to L-890; P-495 to L W-496 to L-890; A-497 to L-890; D-498 to L G-499 to L T-500 to L- 890; P-501 to L C-502 to L G-503 to L P-504 to L G-505 to L-890; H-506 to L-890; L-507 to L-890; C-508 to L-890; S-509 to L E- 5 1 0 to L-890; G-51 I to L-890; S-512 to L-890; C-513 to L-890; L-514 to L-890, P-515 to L-890; E-516 to L E-517 to L E-518 to L-890; V-519 to L- 890; E-520 to L-890; R-521 to L P-522 to L-890; K-523 to L-890; P-524 to L V-525 to L-890; V-526 to L D-527 to L-890; G-528 to L-890; G- 529 to L-890; W-530 to L-890; A-531 to L-890; P-532 to L , W-533 to L- 890; G-534 to L-890; P-535 to L W-536 to L-890; G-537 to L-890; E-538 to L-890; C-539 to L-890; S-540 to L-890; R-541 to L-890; T-542 to L C- 543 to L-890; G-544 to L-890; G-545 to L , G-546 to L-890, V-547 to L- 890; Q-548 to L-890; F-549 to L-890; S-550 to L-890; H-551 to L R-552 to L-890; E-553 to L-890; C-554 to L-890; K-555 to L-890; D-556 to L-890; P- 557 to L-890; E-558 to L-890; P-559 to L Q-560 to L N-561 to L-890; G-562 to L G-563 to L-890; R-564 to L-890; Y-565 to L C-566 to L- 890; L-567 to L G-568 to L-890; R-569 to L-890; R-570 to L A-571 to L-890; K-572 to L Y-573 to L-890; Q-574 to L S-575 to L-890; C- 576 to L-890; H-577 to L-890; T-578 to L E-579 to L-890; E-580 to L-890; C-581 to L P-582 to L-890; P-583 to L D-584 to L-890; G-585 to L- 890- K-586 to L S-587 to L-890; F-588 to L R-589 to L E-590 to L Q-591 to L Q-592 to L C-593 to L E-594 to L K- 595 to L-890; Y-596 to L-890; N-597 to L-890; A-598 to L-890; Y-599 to L- 890; N-600 to L Y-601 to L-890; T-602 to L-890; D-603 to L-890; M-604 to L D-605 to L G-606 to L N-607 to L-890; L-608 to L L-609 to L-890; Q-610 to L-890; W-611 to L-890; V-612 to L-890; P-613 to L- 890; K-614 to L-890; Y-615 to L-890; A-616 to L-890; G-617 to L-890; V-618 to L S-619 to L P-620 to L R-621 to L D-622 to L-890; R- 623 to L-890; C-624 to L-890; K-625 to L L-626 to L-890; F-627 to L C-628 to L R-629 to L A-630 to L R-631 to L G-632 to L- 890; R-633 to L-890; S-634 to L E-635 to L F-636 to L K-637 to L V-638 to L F-639 to L-890; E-640 to L-890; A-641 to L-890; K- 642 to L V-643 to L-890; 1-644 to L-890; D-645 to L-890; G-646 to L-890; T-647 to L-890; L-648 to L-890; C-649 to L G-650 to L P-651 to L- 890; E-652 to L-890; T-653 to L-890; L-654 to L-890; A-655 to L-890; 1-656 to L-890; C-657 to L V-658 to L-890; R-659 to L-890; G-660 to L Q-661 to L C-662 to L-890; V-663 to L

K-664 to L-890; A-665 to L-890; G- 666 to L C-667 to L-890; D-668 to L H-669 to L-890; V-670 to L- 890- V-671 to L-890; D-672 to L S-673 to L P-674 to L R-675 to L-890; K-676 to L-890; L-677 to L-890; D-678 to L-890; K-679 to L-890; C-680 to L-890; G-681 to L-890; V-682 to L C-683 to L G-684 to L- 890- G-685 to L K-686 to L-890; G-687 to L-890; N-688 to L S-689 to L-890; C-690 to L R-691 to L-890; K-692 to L V-693 to L-890; S- 694 to L G-695 to L-890; S-696 to L L-697 to L T-698 to L-890; P-699 to L T-700 to L-890; N-701 to L Y-702 to L G-703 to L- 890; Y-704 to L-890; N-705 to L-890; D-706 to L-890; 1-707 to L-890; V-708 to L T-709 to L-890; 1-710 to L P-711 to L-890; A-712 to L-890; G- 713 to L-890; A-714 to L T-715 to L-890; N-716 to L-890; 1-717 to L D-718 to L V-719 to L-890; K-720 to L-890; Q-721 to L-890; R-722 to L- 890- S-723 to L-890; H-724 to L-890; P-725 to L-890; G-726 to L-890; V-727 to L Q-728 to L-890; N-729 to L D-730 to L G-731 to L-890; N- 732 to L Y-733 to L-890; L-734 to L A-735 to L L-736 to L-890; K-737 to L T-738 to L-890; A-739 to L-890; D-740 to L G-741 to L-890- Q-742 to L-890; Y-743 to L L-744 to L-890; L-745 to L-890; N-746 to L G-747 to L-890; N-748 to L L-749 to L A-750 to L I- 751 to L S-752 to L-890; A-753 to L-890; 1-754 to L E-755 to L Q-756 to L D-757 to L-890; 1-758 to L-890; L-759 to L-890; V-760 to L- 890; K-761 to L-890; G-762 to L T-763 to L-890; 1- 764 to L-890; L-765 to L-890; K-766 to L-890; Y-767 to L S-768 to L G-769 to L-890; S- 770 to L-890; 1-771 to L-890; A-772 to L-890; T-773 to L-890; L-774 to L-890; E-775 to L R-776 to L-890; L-777 to L-890; Q-778 to L-890; S-779 to L-890; F-780 to L-890; R-781 to L-890; P-782 to L L-783 to L-890; P-784 to L-890; E-785 to L-890; P-786 to L-890; L-787 to L-890; T-788 to L-890; V-789 to L Q-790 to L-890; L-791 to L L-792 to L-890; T-793 to L V- 794 to L-890; P-795 to L-890; G-796 to L-890; E-797 to L-890; V-798 to L-890; F-799 to L-890; P-800 to L-890; P-801 to L K-802 to L-890; V-803 to L- 890; K-804 to L-890, Y-805 to L-890; T-806 to L-890; F-807 to L-890; F-808 to L-890; V-809 to L-890; P-810 to L-890; N-811 to L-890; D-812 to L-890; V- 813 to L D-814 to L F-815 to L S-816 to L-890; M-817 to L Q-818 to L-890; S-819 to L S-820 to L-890; K-821 to L E-822 to L- 890; R-823 to L , A-824 to L-890; T-825 to L-890; T-826 to L-890; N-827 to L 1-828 to L-890; 1-829 to L-890; Q-830 to L P-831 to L-890; L- 832 to L-890; L-833 to L H-834 to L-890; A-83 5 to L Q-83 6 to L to W-837 to L V-838 to L-890; L-839 to L-890; G-840 to L D-841 to L- 890- W-842 to L S-843 to L E-844 to L-890; C-845 to L S-846 to L-890; S-847 to L T-848 to L C-849 to L G-850 to L-890; A- 851 to L-890; G-852 to L-890; W-853 to L Q-854 to L-890; R-855 to L- 890; R-856 to L-890; T-857 to L-890; V-858 to L E-859 to L-890; C-860 to L-890; R-861 to L-890; D-862 to L P-863 to L-890; S-864 to L G- 865 to L Q-866 to L A-867 to L-890; S-868 to L A-869 to L-890; T-870 to L-890; C-871 to L-890; N-872 to L-890; K-873 to L A-874 to L- 890; L-875 to L-890; K-876 to L P-877 to L E-878 to L-890; D-879 to L-890; A-880 to L K-881 to L P-882 to L-890; C-883 to L-890; E- 884 to L-890; S-885 to L of SEQ ID NOA Moreover, C-terminal deletions of the METH2 polypeptide can also be described by the general formula I-n, where n is an integer from 2 to 890 where n corresponds to the position of amino acid residue identified in SEQ ID NOA Preferably, C-terminal deletions of the METH2 polypeptide of the invention shown as SEQ ID NO: 4 include polypeptides comprising the amino acid sequence of residues: M-1 to P-889; M-1 to C-888; M-1 to L M-1 to Q M-1 to S-885; M-1 to E-884; M-1 to C M-1 to P MA to K-881; M-1 to A-880; M-1 to D-879; M-1 to E-878; M-1 to P-877; M-1 to K-876; M-1 to L-875,- M- I to A-874; M- I to K-873; M- I to N-872; M- I to C-871 M- 1 to T-870; M- I to A-869; M- I to S-868; M- I to A-867; M- I to Q-866; M- I to G-865; M- I to S- 864; M-1 to P-863; M-1 to D-862; M-1 to R-861; M-1 to C-860; M-1 to E-859; M-1 to V-858; M-1 to T M-1 to R-856; M-1 to R-855; M-1 to Q-854; M-1 to W-853; M- I to G-852; M- I to A-85 1; M- I to G-850; M- I to C-849; M- I to T-848 M- I to S-847; M- I to S-846; M- I to C-845; M- I to E-844; M- I to S-843; M- I to W-842; M- I to D-84 1; M- I to G-840; M- I to L-83 9; M- I to V-83 8; M- I to W M-1 to Q-836; M-1 to A-835; M-1 to H M-1 to L M-1 to L M-1 to P-83 1; M-1 to Q-830; M-1 to 1-829; M-1 to 1 M-1 to N M-1 to T M-1 to T-825; M-1 to A M-1 to R-823; M-1 to E-822; M-1 to K-821; M-1 to S M-1 to S-819; M-1 to Q-818; M-1 to M-817; M-1 to S- 816; M-1 to F M-1 to D-814; M-1 to V-813; M-1 to D M-1 to N-81 I- M-1 to P-810; M-1 to V M-1 to F-808; M-1 to F-807; M-1 to T-806; M-1 to Y-805; M-1 to K-804; M-1 to V M-1 to K M-1 to P-801; M-1 to P- 800; M- I to F-799; M- I to V-798 M- I to E-797; M- I to G-796; M- I to P-795; M- I to V-794; M- I to T-793; M- I to L M- I to L-79 1; M- I to Q-790; M- I to V-789; M-I to T-788; M- I to L M- I to P M- I to E-785; M- I to P- 784; M-1 to L-783; M-1 to P-782; M-1 to R-781; M-1 to F-780; M-1 to S-779; M-1 to Q-778; M-1 to L-777; M-1 to R-776; M-1 to E-775; M-1 to L M-1 to T-773 - M- I to A-772; M- I to 1-771 M- I to S-770; M- I to G-769; M- I to S- 768- M-1 to Y M-1 to K M-1 to L M-1 to 1 M-1 to T M-1 to G-762; M-1 to K M-1 to V M-1 to L-759; M-1 to 1-758; M-1 to D M-1 to Q-756; M-1 to E-755; M-1 to 1-754; M-1 to A-753; M-1 to S- 752; M-1 to 1-75 L M-1 to A M-1 to L-749; M-1 to N-748; M-1 to G M-1 to N-746; M-1 to L-745; M-1 to L-744; M-1 to Y-743; M-1 to Q M-1 to G-74 1; M- I to D-740 M-I to A-73 9; M- I to T-73 8; M- I to K-73 7; M- I to L-73 6; M- I to A-73 5; M-I to L-73 4; M- 1 to Y-73 3; M- I to N-73 2; M- I to G- 73 1; M- I to D-73 0; M- I to N-729; M- I to Q-72 8; M- I to V-727 - M- I to G-726 M- I to P-725 M- I to H-724 M- I to S-723 M- I to R-722; M- I to Q-721 M- I to K-720; M- I to V-719; M- I to D-718 M- I to 1-717; M- I to N-716; M- I to T- 715; M- I to A-714; M- I to G-713; M- I to A-712; M- I to P-71 I M- I to 1-71 0; M- I to T-709; M-I to V-708; M- I to 1-707; M- I to D-706; M- I to N-705; M- I to Y-704 @- M- I to G-703; M- I to Y-702; M- I to N-701 @- M- I to T-700; M- I to P M-1 to T M-1 to L-697; M-1 to S M-1 to G M-1 to S-694; M-1 to V-693; M-1 to K-692; M-1 to R-691 M-1 to C-690; M-1 to S M-1

to N-688; M-1 to G-687; M-1 to K-686@ M-1 to G M-1 to G M-1 to C-683; M-1 to V-682; M-1 to G-681; M-1 to C M-1 to K-679; M-1 to D- 678; M-1 to L M-1 to K-676; M-1 to R-675; M-1 to P-674; M-1 to S-673; M-I to D-672; M-I to V-67 1; M- I to V-670; M- I to H-669; M- I to D-668; M- I to C-667; M-1 to G-666; M-1 to A M-1 to K M-1 to V-663; MA to C-662; M-1 to Q-661; M-1 to G-660; M-1 to R-659; M-1 to V-658; M-1 to C- 657; M-1 to 1-656; M-1 to A-655; M-1 to L-654; M-1 to T M-1 to E-652; M-1 to P-651; M-1 to G M-1 to C M-1 to L-648; M-1 to T-647; M-1 to G-646; M- I to D-645; M- I to 1-644; M- I to V-643; M- I to K-642; M- I to A- 641- M-1 to E M-1 to F M-1 to V M-1 to K M-1 to F M-1 to E-635; M-1 to S M-1 to R-633; MA to G-632; M-1 to R-63 1; M-1 to A-63 0; M- I to R-629; M- I to C-628; M- I to F-627; M- I to L-626; M- I to K-625; M-1 to C-624; M-1 to R-623; M-1 to D-622; M-1 to R-621; M-1 to P-62O.- M-I to S-619; M- I to V-618 - M- I to G-617; M- I to A-616; M- I to Y-615; M- I to K-614 M- I to P-613 M- I to V-612 M- I to W-61 1; M- I to Q-6 I 0; M- I to L-609; M-1 to L-608; M-1 to N-607; M-1 to G-606; M-1 to D-605; M-1 to M- 604- M-1 to D-603; M-1 to T-602; M-1 to Y M-1 to N M-1 to Y M- I to A-598; M- I to N-597; M- I to Y M- I to K-595; M- I to E M- I to C-593; M- I to Q-592; M- I to Q-591 M- I to E-590; M- I to R-5 89; M- I to F-588; M-1 to S-587; M-1 to K-586; M-1 to G-585; M-1 to D-584; M-1 to P-583; M-1 to P-582; M-1 to C-581; M-1 to E-580; M-1 to E M-1 to T-578; M-1 to H-577; M-1 to C-576; M-1 to S-575; M-1 to Q-574; M-1 to Y M-1 to K- 572; M-1 to A-571; MA to R M-1 to R-569; M-1 to G M-1 to L-567; MA to C M-1 to Y M-1 to R-564; M-1 to G M-1 to G M-1 to N-561; M-1 to Q-560; M-1 to P-559; M-1 to E-558; M-1 to P-557; M-1 to D- 556- M-1 to K M-1 to C M-1 to E-553; M-1 to R M-1 to H-55 1; M-1 to S-550; M-1 to F-549; M-1 to Q-548; M-1 to V-547; M-1 to G-546; M-1 to G-545, M- I to G-544; M- I to C-543; M- I to T-542; M- I to R-54 1; M- I to S- 540; M-1 to C-539; M-1 to E M-1 to G M-1 to W-536; M-1 to P M-1 to G-534; M-1 to W-533; M-1 to P-532; M-1 to A-53 1; M-1 to W-530; M-1 to G-529; M-1 to G-528; M-1 to D-527; M-1 to V-526; M-1 to V-525; M-1 to P-524; M-1 to K-523; M-1 to P-522; M-1 to R M-1 to E-520; M-1 to V- 519; M-1 to E-518; M-1 to E-517; M-1 to E M-1 to P-515; M-1 to L M-1 to C M-1 to S-512; M-1 to G-51 1; M-1 to E M-1 to S-509; M-1 to C-508; M-1 to L M-1 to H-506; M-1 to G-505; M-1 to P-504; M-1 to G-503; M-1 to C M-1 to P M-1 to T-500; M-1 to G-499; M-1 to D M- I to A-497; M- I to W M- I to P-495; M- I to L-494; M- I to S-493; M- I to G-492; M-1 to N-491; M-1 to K-490; M-1 to T-489; M-1 to H M-1 to C-487; M-1 to L-486; M-1 to P M-1 to E-484; M-1 to A-483; M-1 to G- 482; M- I to D-481 M- I to T-480; M- I to H-479; M- I to C-478; M- I to W-477; M-1 to L M-1 to Q M-1 to A M-1 to C-473; M-1 to V-472; M-1 to D-471; M-1 to Q-470; M-1 to A-469; M-1 to S-468; M-1 to T-467; M-1 to N- 466- MA to P-465; M-1 to C M-1 to H M-1 to R M-1 to F-461; M-1 to D-460; M-1 to P-459; M-1 to G-458; M-1 to F-457; M-1 to 1-456; M-1 to Q-455; M-1 to R-454; M-1 to C-453; M-1 to Q-452; M-1 to Q-451; M-1 to D M-1 to L-449; MA to Q-448; M-1 to Y M-1 to L-446; M-1 to A- 445; M- I to M M- I to R-443 - M- I to G-442; M- I to P-44 1; M-I to L-440; M-1 to G-439; M-1 to T-438; MA to P-437; M-1 to L M-1 to P M-1 to L-43 4; M- I to A-43 3; M- I to A-43 2; M- I to G-43 1; M- I to P-43 0 M- I to A- 429; M- I to D-428; M- I to L-427; M- I to L-426; M- I to C-425; M- I to D-424; M-1 to G-423; M-1 to H M-1 to G-421; MA to G M-1 to D M-1 to L-418 - M- I to L-417; M- I to E M- I to T-415; M- I to L-414; M- I to Y- 413; M- I to M-412; M- I to A-41 I - M- I to S-41 0; M- I to C-409; M- I to P-408 M- I to S-407; M- I to W-406 M- I to P-405; M- I to L-404; M- I to T-403 M- I to Q-402; M-1 to N-401; M-1 to L-400; M-1 to H-399; M-1 to V-398; M-1 to F-397; M-1 to L-396; M-1 to P-395; M-1 to A-394; M-1 to M-393; M-1 to V- 392- M-1 to H-391; M-1 to H-390; M-1 toK M-1 to G-388; M-1 to M-387; M-1 to P M-1 to G M-1 to F-384; M-1 to L-383; M-1 to R-382; M-1 to T-381; M-1 to C-380; M-1 to P-379; M-1 to K-378; M-1 to S M-1 to D- 376; M-1 to D-375; M-1 to H M-1 to P-373; M-1 to M-372; M-1 to S-371; M-1 to L-370; M-1 to V M-1 to H-368; M-1 to G M-1 to L M-1 to E-3 65; M- I to H-3 64; M- I to A-3 63 - M- I to L-3 62; M- I to T-3 6 1; M- I to H-360; M-1 to A-359; M-1 to A M-1 to Q-357; M-1 to L-356; M-1 to G-355; M-1 to E M-1 to D M-1 to E M-1 to 1-35 1; M-1 to V-350; M-1 to S-349; M- I to C-348; M- I to S-347; M- I to K M- I to N-345; M- I to P- 344; M-1 to D-343; M-1 to C M-1 to 1-341; M-1 to T M-1 to G-339; M-1 to 1-338; M-1 to D M-1 to A M-1 to V-335; M-1 to G-334; M-1 to L M-1 to T M-1 to D-331; M-1 to C-330; M-1 to L M-1 to G- 328- M-1 to E-327; M-1 to Q-326; M-1 to G M-1 to C M-1 to F-323; M-1 to N M-1 to Q-321; M-1 to R-320; M-1 to T-319; M-1 to L-318; M-1 to L-317; M- I to 1-316; M- I to A-315; M- I to T-314; M- I to D-313; M- I to Y- 1 5 312; M- I to H-31 I - M- I to E-3 I 0; M- I to P-3 09; M- I to H-3 08; M- I to R-3 07- M- I to D-3 06; M- I to S-3 05; M- I to P-3 04; M- I to Q-3 03; M- I to N-3 02; M- I to F M-1 to R-300; M-1 to R M-1 to Q M-1 to W M-1 to N M-1 to C-295; M-1 to F-294; M-1 to N M-1 to R-292; M-1 to L- 291; M-1 to T-290; M-1 to L-289; M-1 to G M-1 to G-287; M-1 to N M-1 to D-285; M-1 to S M-1 to V M-1 to E-282; MA to P M-1 to G-280; M-1 to W-279; M-1 to K-278; M-1 to E-277; M-1 to D-276; M-1 to E-275; M-1 to V-274; M-1 to 1-273; M-1 to L MA to V-271; MA to K- 270; M- I to V-269; M- I to V-268 - M- I to M-267; M- I to L-266; M- I to N-265; M-1 to 1 M-1 to S-263; M-1 to N-262; M-1 to K-261; M-1 to 1-260; M-1 to S-259; M-1 to P-258; M-1 to H-257; M-1 to K MA to Y M-1 to I- 254; M-1 to R M-1 to A-252; M-1 to A-251 M-1 to V-250; M-1 to S-249; M-1 to M M-1 to L M-1 to T MA to L M-1 to 1 M-1 to H M-1 to N M-1 to Q-241; M-1 to L-240; M-1 to D-239; MA to A-238; M-1 to G-237; M-1 to Y M-1 to F MA to A-234; M-1 to A- 233; M-1 to M M-1 to S-231; M-1 to A-230; M-1 to D-229; M-1 to A-228; M- I to V-227; M- I to L-226 M- I to L-225; M- I to T-224; M- I to E-223; M- I to V M- I to F-22 1; M- I to R-220; M-I to A-219; M- I to E-218 M- I to S- 217 M- I to V-216; M- I to F-215 M- I to R-214; M- I to

K-213 M- I to T-212; M- I to R-21 I M- I to S-21 0; M- I to T-209 M- I to A-208; M- I to G-207; M- I to L-206; M-1 to P-205; M-1 to P M-1 to P-203; M-1 to P-202; M-1 to E- 201- M-1 to S-200; M-1 to A-199; M-1 to G-198; M-1 to E M-1 to A-196; M-1 to E M-1 to E-194; M-1 to E-193; M-1 to Q-192; MA to S-191; M-1 to E M-1 to E-189; MA to E M-1 to S-187; M-1 to D-186; M-1 to E-185; M-1 to Q M-1 to H-183; M-1 to D M-1 to G M-1 to R M-1 to E-179; M-1 to Q M-1 to R M-1 to Q M-1 to G M-1 to E-174; M-1 to G M-1 to T-172; M-1 to E-171; M-1 to V-170; M-1 to E- 169- M-1 to W-168; M-1 to E M-1 to P M-1 to G-165; M-1 to R-164; M-1 to P-163; M-1 to L-162; M-1 to P-161; M-1 to R-160; M-1 to A-159; M-1 to G-1 58; M-1 to A-1 57; M-1 to P-1 56; M-1 to G-1 55; M-1 to W-1 54; M-1 to t5 R-153; M-1 to Q-152; M-1 to L-151; M-1 to R-150; M-1 to H-149; M-1 to P-148; M-1 to Q M-1 to A-146; M-1 to L-145; M-1 to S-144; M-1 to G M-1 to G M-1 to A M-1 to G-140; M-1 to Q-139; M-1 to P-138; M-1 to Q M-1 to 1-136; M-1 to T M-1 to F M-1 to E-133; M-1 to E- 132- M-1 to G-13 I- M-1 to D M-1 to L-129; M-1 to L-128; M-1 to F M-1 to S-126; M-1 to G-125; M-1 to S-124; M-1 to L-123; M-1 to G-122; M-1 to R M-1 to C-120; M-1 to L-1 19; M-1 to S-1 18; M-1 to V-1 17; M-1 to A- 116; M-1 to A-1 15- M-1 to L-1 14- M-1 to S-1 13; M-1 to E-1 12; M-1 to P-1 I I- M-1 to E-1 10- M-1 to G-109; M-1 to N-108; M-1 to V-107; M-1 to T-106; M-1 to G-105; M-1 to S-104; M-1 to F M-1 to F-102; M-1 to C-101 M-1 to G-100; M-1 to R M-1 to L M-1 to G-97; M-1 to R-96; MA to E M-1 to G M- I to G-93 M- I to T-92; M- I to A- 91 M- I to R-90; M- I to G M-1 to S-88; MA to G M-1 to G-86; M-1 to L-85; M-1 to R-84; M-1 to E- M- I to 1 M- I to K-81 M- I to F-80; M- I to E-79; M- I to P-78 M- I to A MA to L-76; M-1 to F M-1 to S-74; M-1 to D-73; M-1 to D-72; M-1 to P M-1 to A-70; M-1 to L M-1 to R M-1 to L-67; M-1 to V-66; M- 1 to F-65; M- I to G M- I to K-63; M- I to G-62; M- I to F-6 1; M- I to A-60; M-1 to S M-1 to L-58; M-1 to H-57; MA to L-56; MA to A-55; MA to L-54; M- 1 to E-53; M- I to G-52; M- I to A-5 1; M- I to S-50; M- I to G-49; M- I to P MA to L MA to R-46; MA to T MA to P MA to V-43; M-1 to V-42; M- I to L-4 1; M- I to E-40; M- I to S-3 9; M- I to A-3 8; M- I to Q-3 7; M- I to G-3 6; M-I to G-3 5; M- I to A-34; M- I to A-3 3; M- I to P-3 2 M- I to R-3 1; M- I to A-3 0; M- I to P-29; M- I to A-28; M- I to G-27; M- I to

R-26; M- I to A- 25; M- I to L-24; M- I to P-23; M- I to L-22; M- I to L-2 1; M- I to L-20; M- I to L-19; M-1 to L-18; M-1 to L-17; M-1 to L M-1 to L-15; M-1 to L-14; M-1 to F M-1 to P M-1 to L-1 1; M-1 to W-10; M-1 to R-9; M-1 to P-8; M-1 to A-7; of SEQ ID NOA Preferably, any of the above listed N-or C-terminal deletions can be combined to produce a N- and C-terminal deleted NIETH2 polypeptide.

The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of SEQ ID NO:2 or SEQ ID NOA, where n and in are integers as described above.

Also preferred are METHI or MIETH2 polypeptide and polynucleotide fragments characterized by structural or functional domains. Preferred embodiments of the invention include fragments that comprise alpha-helix and alpha-helix forming regions ("alpha-regions"), beta-sheet and beta-sheet-forming regions ("beta-regions"), turn and turn-forming regions ("turn-regions"), coil and coil-forming regions ("coil-regions"), hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. As set out in the Figures, such preferred regions include Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha and beta amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions, and Jameson-Wolf high antigenic index regions. Polypeptide fragments of SEQ ID NO:2 falling within conserved domains are specifically contemplated by the present invention, (See Figures I 0 & I I and Tables I & 2.) Moreover, polynucleotide fragments encoding these domains are also contemplated.

Other preferred fragments are biologically active METHI or METI-12 fragments. Biologically active fragments are those exhibiting activity similar, but ily identical, to an activity of the METHI or METI-12 polypeptide.

not necessari The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

However, many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases. Some of these sequences are related to SEQ ID NO: I or SEQ ID NO-3 and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would be cumbersome. Accordingly, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide

sequence described by the general formula of a-b, where a is any integer between I to 93 6 of SEQ ID NO - 1, b is an integer of 15 to 950, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO- 1, and where the b is greater than or equal to a + 14. Moreover, preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between I to 876 of SEQ ID NO:3, b is an integer of 15 to 890, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO: 3, and where the b is greater than or equal to a + 14.

Epitopes & Antibodies In the present invention, "epitopes" refer to METHI or METI-12 polypeptide fragments having antigenic or immunogenic activity in an animal, especially in a human. A preferred embodiment of the present invention relates to a METHI or METH2 polypeptide fragment comprising an epitope, as well as the polymicleotide encoding this fragment. A region of a protein molecule to which an antibody can bind is defined as an "antigenic epitope." In contrast, an "immunogenic epitope" is defined as a part of a protein that elicits an antibody response. (See, for instance, Geysen el aL, Proc. NatL Acad. Sci. USA 81:3998- 4002 (1983).) Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten, R. A., Proc. NatL Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,21 1.) In the present invention, antigenic epitopes preferably contain a sequence of at least seven, more preferably at least nine, and most preferably between about 15 to about 30 amino acids. Antigenic epitopes are useful to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. (See, for instance, Wilson el aL, Cell 37:767-778 (1984); Sutcliffe, J. G. et aL, Science 219:660-666 (1983).) Similarly, immunogenic epitopes can be used to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et aL, supra; Wilson et al., supra- Chow, M. el al., Proc. Naff A cad. Sci. USA 82:91 0- 914- and Bittle, F. J. et aL, J Gen. Virol. 66:2347- 2354 (1985).) A preferred immunogenic epitope includes the secreted protein. The immunogenic epitopes may be presented together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse) or, if it is long enough (at least about 25 amino acids), without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting.) Using DNAstar analysis, SEQ ID NO:2 was found antigenic at amino acids: 2-14,32-44, 47-60, 66-78, 87-103, 109-118, 146-162@ 168-1807 183-219@ 223 284@ 296 3341341-354 @ 357-376,392-399,401-410) 418-429,438-454,456-471,474-488,510-522,524-538 @ 550-561@ 565-626,630-6437 659-6717 679 749@ 784-804@ 813-820@ 825-8327 845- 854@ 860-894@ 899- 917, 919-924 and 928 Thus, these regions could be used as epitopes to produce antibodies against the protein encoded by METHI cDNA.

Using DNAstar analysis, SEQ ID NO:4 was found antigenic at amino acids: 26-38, 45-52, 69-76, 80-99, 105-113, 129-136, 138-217, 254-263, 273- 289,294-313,321- 331,339-3567 371 4271438-443@ 459-471@ 479-505@ 507-526,535-5467 550-607,615- 640, 648-653@ 660-667@ 669-68 1@ 683-7047 717- 732@ 737-743@ 775-787, 797-804 @ 811-825, 840-867 and 870 Thus, these regions could be used as epitopes to produce antibodies against the protein encoded by METH2 cDNA.

As used herein, the term "antibody" (Ab) or "monoclonal antibody" (Mab) is meant to include intact molecules as well as antibody fragments (such as, for example, Fab and F(ab')2 fragments) which are capable of specifically binding to protein. Fab and F(ab')2 fragments lack the Fc fragment of intact antibody, clear more rapidly from the circulation, and may have less non-specific tissue binding than an intact antibody. (Wahl et al., J Nucl. Med. 24316-325 (1983).) Thus, these fragments are preferred, as well as the products of a FAB or other immunoglobulin expression library. Moreover, antibodies ofthe present invention include chimeric, single chain, and humanized antibodies.

Fusion Noteins Any N[ETHI or MIETH2 polypeptide can be used to generate fusion proteins. For example, the METHI or METH2 polypeptide, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the METHI or METH2 polypeptide can be used to indirectly detect the second protein by binding to the METHI or METH2. Moreover, because secreted proteins target cellular locations based on trafficking signals, the METHI or METH2 polypeptides can be used as a targeting molecule once fused to other proteins.

Examples of domains that can be fused to METHI or METH2 polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

Moreover, fusion proteins may also be engineered to improve characteristics of the METHI or NIIETH2 polypeptide.

For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N- terminus of the METHI or METI-12 polypeptide to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties may be added to the METHI or METI-12 polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the METHI or METH2 polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Moreover, METHI or NIIETH2 polypeptides, including fragments, and specifically epitopes, can be combined with parts of the constant domain of immunoglobulins (IgG), resulting in chimeric polypeptides. These fusion proteins facilitate purification and show an increased half-life in vivo. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains ofthe constant regions of the heavy or light chains of mammalian immunoglobulins. (EP A 394,827,- Traunecker et al., Nature 331:84-86 (1988).) Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone.

(Fountoulakis et al., J. Biochern. 270:3958-3964 (1995).) Similarly, EP-A-0 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations, In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).) Moreover, the NIETHI or METH2 polypeptides can be fused to marker sequences, such as a peptide which facilitates purification of METHI or METH2.

In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 913 1 1), among others, many ofwhich are commercially available. As described in Gentz et al., Proc. Nall. Acad. Sci. USA 86:8ZI-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein.

(Wilson et al., Cell 37:767 (1984).) Thus, any of these above fusions can be engineered using the NETHI or METH2 polynucleotides or the polypeptides.

Biological Activities of METHI or METH2 METHI or METH2 polynucleotides and polypeptides can be used in assays to test for one or more biological activities. If METHI or METH2 polynucleotides and polypeptides do exhibit activity in a particular assay, it is likely that METH I or METH2 may be involved in the diseases associated with the biological activity. Therefore, METHI or METH2 could be used to treat the associated disease.

Immune Activity N/1ETH1 or METI-12 polypeptides or polynucleotides may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells.

Immune cells develop through a process called hematopoiesis, producing myelold (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious.

Moreover, METHI or METI-12 polynucleotides or polypeptides can be used as a marker or detector of a particular immune system disease or disorder.

METHI or METI-12 polynucleotides. or polypeptides may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. METHI or METH2 polypeptides or polynucleotides could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia,

dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, METH I or METI-12 polypeptides or polynucleotides can also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, METH I or METI-12 polynucleotides or polypeptides could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, METH I or METI-12 polynucleotides or polypeptides that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting, important in the treatment of heart attacks (infarction), strokes, or scarring.

METHI or METH2 polynucleotides or polypeptides may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of METHI or METH2 polypeptides or polynucleotides that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoinimune disorders.

Examples of autoimmune disorders that can be treated or detected by METH I or METH2 include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves'Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Peniphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoinimune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoinimune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by METH I or METH2 polypeptides or polynucleotides. Moreover, METH I or METH2 can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

METH I or METH2 polynucleotides or polypeptides may also be used to treatand/orpreventorganrejectionorgraft-versus-hostdisease(GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues.

The administration of METHI or METH2 polypeptides or polynucleotides that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVM.

Similarly, METH I or METH2 polypeptides or polynucleotides may also be used to modulate inflammation. For example, WTHI or NETH2 polypeptides or polynucleotides may inhibit the proliferation and differentiation of cells involved in an inflammatory response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL) Hyperproliferative Disorders METHI or METH2 polypeptides or polynucleotides can be used to treat or detect hyperproliferative disorders, including neoplasms. NMTHI or METH2 polypeptides or polynucleotides may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, METHI or WTH2 polypeptides or polynucleotides may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated.

This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

-too- Examples of hyperproliferative disorders that can be treated or detected by METHI or NIETH2 polynucleotides or polypeptides include, but are not limited to neoplasms located in the: abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by METHI or METH2 polynucleotides or polypeptides. Examples of such hyperproliferative disorders include, but are not limited to- to hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

li@feetious Disease METH I or METH2 polypeptides or polynucleotides can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, METH I or METH2 polypeptides or polynucleotides may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by METH I or METH2 polynucleotides or polypeptides. Examples ofviruses, include, but are not limited to the following DNA and RNA viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e,g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza), Papovaviridae, Parvoviridae, Picomaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-1, HTLV-11, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox , hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. METHI or N/1ETH2 polypeptides or polynucleotides can be used to treat or detect any of these symptoms or diseases.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by METHI or METH2 polynucleotides or polypeptides include, but not limited to, the following Gram-Negative and Gram-positive bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, Enterobacteriaceae (Klebsiella, Salmonella, Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Menigococcal), Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus, Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, and Staphylococcal.

These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning, Typhoid, pneumonia, Gonorrhea, meningitis, Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, Regeneration METHI or METH2 polynucleotides or polypeptides can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues.

(See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue

damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteocarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vascular (including vascular endothelium), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue, Preferably, regeneration occurs without or decreased scarring. Regeneration also may include anglogenesis.

Moreover, METHI or METH2 polynucleotides or polypeptides may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. METH I or METH2 polynucleotides or polypeptides of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using METHI or METH2 polynucleotides or polypeptides to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stoke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resultingfrom chemotherapy or othermedical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the METHI or METH2 polynucleotides or polypeptides.

Chemotaxis METH I or METH2 polynucleotides or polypeptides may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

METHI or METH2 polynucleotides or polypeptides may increase chemotaxic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. As a chemotactic molecule, WTHI or N1ETH2 could also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that METHI or NIETH2 polynucleotides or polypeptides may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, NIETHI or NffiTH2 polynucleotides or polypeptides could be used as an inhibitor of chemotaxis.

Binding Activity METH I or METH2 polypeptides may be used to screen for molecules that bind to METH I or METH2 or for molecules to which METH I or NMT142 binds.

The binding of METHI or METH2 and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the METHI or N/IETH2 or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors),or small molecules.

Preferably, the molecule is closely related to the natural ligand of METHI or METI-12, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan el al., Current Protocols in Immunology](2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which NIETHI or METH2 binds, or at least, a fragment of the receptor capable of being bound by METHI or N4ETH2 (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express METH I or METH2, either as a secreted protein or on the cell membrane. Preferred cells include cells from mammals, yeast, Drosophila, or

E. coli. Cells expressing METH I or NIETH2(or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either METH I or METH2 or the molecule.

The assay may simply test binding of a candidate compound toMETH I or METH2, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to NIIETHI or METH2.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing METHI or METH2, measuring MIETH I or METH2/molecule activity or binding, and comparing the METH I or METH2/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure METHI or NMTH2 level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure NETHI or METH2 level or activity by either binding, directly or indirectly, to METH I or METH2 or by competing with METH I or METH2 for a substrate.

All of these above assays can be used as diagnostic or prognostic markers, The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the, METH I or METH2 molecule. Moreover, the assays can discover agen ts which may inhibit or enhance the production of METH I or N/IETH2 from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to METHI or METH2 comprising the steps of: (a) incubating a candidate binding compound with METHI or METH2; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of (a) incubating a candidate compound with METH I or METH2, (b) assaying a biological activity, and (b) determining if a biological activity of METHI or METH2 has been altered.

Other Activities METH I or METH2 polypeptides or polynucleotides may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

MIETHI or METH2 polypeptides or polynucleotides may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, METH I or METH2 polypeptides or polynucleotides may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

METHI or METH2 polypeptides or polynucleotides may be used to change a mammal's mental state or physical state by influencing biorhythms, circadian rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

NIETHI or MIETH2 polypeptides or polynucleotides may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Cancer Diagnosis and Prognosis It is believed that certain tissues in mammals with cancer express significantly diminished levels of the METHI or METH2 protein and m-RNA encoding the NIETHI or WTH2 protein when compared to a corresponding "standard" mammal, i.e., a mammal of the same species not having the cancer.

Further, it is believed that diminished levels of the METH I or METH2 protein can be detected in certain body fluids (e.g., sera, plasma, urine, and spinal fluid) from mammals with cancer when compared to sera from mammals of the same species not having the cancer. Thus, the invention provides a diagnostic method useful during tumor diagnosis, which involves assaying the expression level of the gene encoding the METH I protein in mammalian cells or body

fluid and comparing the gene expression level with a standard MLETHI gene expression level, whereby a decrease in the gene expression level under the standard is indicative of certain tumors. The invention also provides a diagnostic method useful during tumor diagnosis, which involves assaying the expression level of the gene encoding the NMTH2 protein in mammalian cells or body fluid and comparing the gene expression level with a standard MLETH2 gene expression level, whereby a decrease in the gene expression level under the standard is indicative of certain tumors.

Where a tumor diagnosis has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting diminished METH I or METH2 gene expression will experience a worse clinical outcome relative to patients expressing the gene at a lower level.

By "assaying the expression level of the gene encoding the METHI or METH2 protein" is intended qualitatively or quantitatively measuring or estimating the level of the METH I or METH2 protein or the level of the mRNA encoding the METH I or NffiTH2 protein in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the METHI or METH2 protein level or mRNA level in a second biological sample).

Preferably, the WTH I or METH2 protein level or mRNA level in the first biological sample is measured or estimated and compared to a standard METH I or METH2 protein level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the cancer. As will be appreciated in the art, once a standard METHI or METH2 protein level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source which contains WTHI or METH2 protein or mRNA. Biological samples include mammalian body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) which contain secreted mature METHI or MIETH2 protein, and adrenal, thyroid, stomach, brain, heart, placenta, lung, liver, muscle, kidney, pancreas, testis and ovarian tissue (for METH 1) and prostate, small intestine, colon, brain and lung tissue (for NIETH2), The present invention is useful for detecting cancer in mammals. in particular the invention is useful during diagnosis of the of following types of cancers in mammals: breast, ovarian, prostate, liver, lung, pancreatic, colon, and testicular. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Total cellular RNA can be isolated from a biological sample using the single-step guanidinium-thiocyanate-phenol-chloroform method described in Chomczynski and Sacchi, AnaL Biochem. 162:156-159 (1987). Levels of niRNA encoding the METH I or NffiTH2 protein are then assayed using any appropriate method. These include Northern blot analysis (Harada el al., Cell 63:303-312 (1990)), SI nuclease mapping (Fujita et al., Cell 49:357- 367 (1987)), the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR) (Makino et aL, Technique 2:295-301 (1990)), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

Assaying METHI or METH2 protein levels in a biological sample can occur using antibody-based techniques. For example, NMTH I or METH2 protein expression in tissues can be studied with classical immunohistological methods (Jalkanen, M., et aL, J. Cell. BioL 101:976-985 (1985); Jalkanen, M., et aL, J.

Cell. Biol. 105:3087-3096 (1987)).

Other antibody-based methods useful for detecting WTHI or METH2 protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA).

Suitable labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine (1?1, "11), carbon ("C), sulfur CS), tritium (3 H), indium ("'In), and technetium ("Tc), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Modes qf administration It is recognized than an increase in the vascular supply plays a central role in tumor progression and metastasis; therefore, inhibitors of anglogenesis can prove effective as adjuvant therapy for cancer patients. Some of the currently recognized angiogenic suppressors are poor candidates for systemic treatment due to severe collateral effect. The present inventors have found that METH I and METH2 are potent inhibitors of angiogenesis both in vitro and in vivo. The advantage of METHI and METHI is that these inhibitors are normally

associated with suppression of physiological angiogenesis- therefore, they offer lack of toxicity and endothelial specificity over other angiogenic inhibitors. Furthermore, METHI and NIETH2 present a restricted pattern of expression providing a ible advantage on organ specificity.

possi Accordingly, the polypeptides of the present invention may be employed to treat cancer. The NffiTHI and NffiTH2 polypeptides of the present invention can also be used to treat individuals with other disorders that are related to angiogenesis, including abnormal wound healing, inflammation, rheumatoid arthritis, psoriasis, endometrial bleeding disorders, diabetic retinopathy, some forms of macula degeneration, hemangiomas, and arterial-venous malformations.

Thus, the invention provides a method of inhibiting anglogenesis in an individual comprising administering to such an individual a pharmaceutical composition comprising an effective amount of an isolated METHI polypeptide of the invention, effective to increase the METHI activity level in such an individual. The invention also provides a method of inhibiting angiogenesis in an individual comprising administering to such an individual a pharmaceutical composition comprising an effective amount of an isolated METH2 polypeptide of the invention, effective to increase the METH2 activity level in such an individual.

METHI polypeptides which may be used to inhibit angiogenesis in this manner include: NMTH I polypeptide encoded by the deposited cDNA including the leader; the mature NMTHI polypeptide encoded by the deposited the cDNA minus the leader (i.e., the mature protein)- a polypeptide comprising amino acids about I to about 950 in SEQ ID NO:2; a polypeptide comprising amino acids about 2 to about 950 in SEQ ID NO:2; a polypeptide comprising amino acids about 29 to about 950 in SEQ ID NO:2; a polypeptide comprising amino acids about 30 to about 950 in SEQ ID NO:2-, a polypeptide comprising the metalloprotease domain of WTHI, amino acids 235 to 459 in SEQ ID NO:2; a polypeptide comprisingthe disintegrindomainofMETHI, amino acids460to 544 in SEQ ID NO:2; a polypeptide comprising the first TSP-like domain of METHI, amino acids 545 to 598 in SEQ ID NO:2; a polypeptide comprising the second TSP-like domain of METHI, amino acids 841 to 894 in SEQ ID NO:2; a polypeptide comprising the third TSP-like domain of METH I, amino acids 895 to 934 in SEQ ID NO:2; a polypeptide comprising amino acids 536 to 613 in SEQ ID NO - 2; and a polypeptide comprising amino acids 549 to 5 63 in SEQ ID NO: 2.

METH2 polypeptides which may be used to inhibit angiogenesis in this manner include: the METH2 polypeptide encoded by the deposited cDNA including the leader; the mature METH2 polypeptide encoded by the deposited the cDNA minus the leader (L e., the mature protein) a polypeptide comprising amino acids about I to about 890 in SEQ ID NO-4; a polypeptide comprising amino acids about 2 to about 890 in SEQ ID NOA; a polypeptide comprising amino acids about 24 to about 890 in SEQ ID NOA- a polypeptide comprising amino acids about 112 to about 890 in SEQ ID NOA; a polypeptide comprising the metalloprotease domain of METH2, amino acids 214 to 439 in SEQ ID NOA; a polypeptide comprising the disintegrin domain of METH2, amino acids 440 to 529 in SEQ ID NOA; a polypeptide comprising the first TSP-like domain of METH2, amino acids 530 to 583 in SEQ ID NOA; a polypeptide comprising the second TSP-like domain of METH2, amino acids 837 to 890 in SEQ ID NOA; a polypeptide comprising amino acids 280 to 606 in SEQ ID NOA; and a polypeptide comprising amino acids 529 to 548 in SEQ ID NOA.

As a general proposition, the total pharmaceutically effective amount of METH I or METH2 polypeptide administered parenterally per dose will be in the range of about I @tg/kg/day to I 0 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0. 0 1 and I mg/kg/day for the polypeptide. If given continuously, the METH I or METH2 polypeptide is typically administered at a dose rate of about I @tg/kg/hour to about 50 [tg/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed.

Pharmaceutical compositions containing the METHI or METH2 of the invention may be administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), bucally, or as an oral or nasal spray. By "pharmaceutically acceptable carrier" is meant a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Chromosome Assays The nucleic acid molecules of the present invention are also valuable for I 0 chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

In certain preferred embodiments in this regard, the cDNA herein disclosed is used to clone genomic DNA of a METH I or METH2 protein gene. This can be accomplished using a variety of well known techniques and libraries, which generally are available commercially. The genomic DNA then is used for in situ chromosome mapping using well known techniques for this purpose.

In addition, in some cases, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes.

Fluorescence in situ hybridization ("FISH") of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with probes from the cDNA as short as 50 or 60 bp. For a review of this technique, see Verma el al., Human Chromosomes: A Allanual Qf Basic Techniques, Pergamon Press, New York (1988), Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance In Man, available on-line through Johns Hopkins University, Welch Medical Library. The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

1 5 Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Examples Example 1: Identification and cloning of METHI and METH2 To search for novel genes with TSP-like domains, a large human cDNA database consisting of approximately 900,00 expressed sequence tags (ESTs) was screenedforsequenceshomologoustothesecon. Several ESTs were predicted to encode proteins with TSP-like domains. Two cDNA clones originated from human heart and lung libraries were further sequenced and chosen for functional analysis.

The amino-terminal end of METHI was obtained using 5' rapid amplification of cDNA ends (RACE) PCR technique (Marathon cDNA amplification kit, Clontech) according to manufacturer instructions, The ammo- terminal end of METH2 was obtained partially through 5'RACE PCR and later confirmed and completed by genomic screening. For the genomic screen, BAC clones (Genome Systems) were initially identified by PCR. Positive BAC clones containing 150-200bp of sequence were subsequently subcloned into pGEM vector as small fragments and sequenced.

Analysis and comparison of the deduced amino acid sequence with the GenBank, ENIBL and SwissProt databases suggested that these genes belong to a new family of metalloproteases with homology to the reprolysin family in their NH2- terminal end and with several TSP-like motifs in the COOH-terminal end.

These cDNAs were named METH I and METH2; ME, for metalloprotease and TH, for thrombospondin. The mouse homologue of METHI was identified and named ADAMTSI (Kuno, K., elaL, J BW Chem. 272 562 (1997)). Direct comparison of the human and mouse sequences revealed a high level of conservation (83.4% amino acid identity). Thus far no homologues for METH2 have been identified.

Interestingly, a recently identified protein named pNPI (procollagen I N-proteinase- (Colidge, A., etaL, Proc. NatL Acad. Sci. USA 94:2374-2379 (1997)) showed a striking sequence and structural similarity to METHI and METH2 (Figure 3). As the novel proteins described here, pNPI also contains metalloproteinase (reprolysin subfamily) and TSP domains at the carboxy-terminal end. Although the sequence for pNPI is of bovine origin, sequence alignment

revealedidenticalstructuralfeatures. TheaminoacidsimilaritybetweenmETHI and METH2 is 51.7%, and between METH I or METH2 and pNPI the homology is lesser 33.9% and 36.3%, respectively.

Sequence analysis showed that the ORF of METHI and METH2 coded for proteins of 950 and 890 amino acids, respectively. In all three proteins, the NH2 terminal end contains a putative signal peptide followed by another putative transmembrane domain around amino acid 300, deduced from the hydrophilicity plots. It is not clear whether these proteins are bound to the membrane.

However, given preliminary data, it is more likely that this second transmembrane domain will consist of a hydrophobic pocket and that METH 1, N4ETH2 and pNPI are in fact secreted proteins. The NI terminal end past the signal peptide has homology to the superfamily of zinc metalloproteases and can be subdivided in a prodomain, a metalloprotease domain, and a cysteine-rich region.

The double underlined sequence in METHI and METH2 in Figure 3 localized at the boundary between the prodomain and the metalloprotease domain, are potential cleavage sites for mammalian subtilisins, such as furins (Barr, 1991).

Proteolytical processing occurs in SVMPs to yield soluble metalloproteases and disintegrins (Bjarnason, J.B. & Fox, JW., Methods Enzymol. 248:345-368 (1995)) andhasalsobeendetectedinsomeADAMs(reviewedbyWolsberg,T.G.

& White, J.M., Developmental Biology 180:389-401 (1996)). At this point, preliminary experiments suggest that proteolytical processing occurs, at least in METHL Additionally, both NIETHI and METH2 present a Zn2' -binding site (dotted line in Figure 3) that is presumed to be catalytically active due to the conservation of certain functionally important amino acids (Rawlings, N.D. & Barrett, A.J., Methods Enzymol. 248:183-228 (1995)) suggesting that these proteins may be active proteases. Following the metalloprotease domain, there is a cysteine-rich region which contains two putative disintegrin loops (Wolsberg, T.G. & White, J.M., Developmental Biology 180:3 89-401 (1996)) (marked by arrows in Figure 3). Disintegrin domains are found within the superfamily of metalloproteases in snake venom metalloproteases (SVMPs) and ADAMs (mammalian proteins containing a disintegrin and a metalloprotease domain) and have a possible function inhibiting binding of integrins to their ligands in SVMPs.

Conversely, the ADAM-disintegrin-like domain, as part of membrane anchored proteins, may promote rather than disrupt, cell-cell interactions (Wolsberg, T. G.

& White, J.M., Developmental Biology 180:389-401 (1996)). The TSP-like domains are located in the COOH-half of NffiTH I and METH2 proteins. METH I contains two conserved TSP domains separated by a spacer region with unknown function, and a subdomain with less homology, and only 5 cysteines, following the second anti-angiogenic region. METH2 contains two TSP domains separated by the spacer region. The alignment of the TSP-like domains of METHI and METI-12 with those of TSP I and TSP2 are shown in Figure 5. The homology varies between 19.2% to 52% amino acid similarity among all the TSP repeats.

The cysteines, numbered I to 6, and the tryptophans, labeled by asterisks, are highly conserved.

Southern blot of human genomic DNA revealed the presence of N.MTH I and METH2 in the genome. METHI and METH2 probes revealed bands of different size suggesting that they are transcribed from different genes.

The consensus sequence for the type I repeats includes 16 residues with 6 perfectly conserved cysteines. Typically it begins with the sequence motif WSXWS (SEQ ID NO: 82) that has also been shown to bind to heparin (Guo, N., el al., J Biol. Chem. 267:19349-19355 (1992)). The affinity of this region to heparin has been proposed to the part of the anti-angiogenic activity of TSP-1 (Guo, N., el al., J Peptide Res. 49 (1997)). Among the five members of the TSP family of proteins, only TSP- I and TSP-2 inhibit angiogenesis and contain the type I repeats (Tolsma, S.S., et al., J Cell. Biol. 122:497-511 (1993); Kyriakides, T.R., et al., J Cell Biol. 140:419-430 (1998)). The type I or properdin repeats were probably added to the precursor of TSP I and 2 by exon shuffling between 500 and 900 years ago (Adams, J., el al., The Thrombospondin Gene Family, I Ed. Molecular Biology Intelligence Unit (Springer, Ed.), R. G. Landes Company, Germany (1995)). It is likely that the acquisition of this domain provided the precursor of TSPI and TSP2 with functions, such as regulation of new vessel formation. More recently, BAI- I (brain anglogenic inhibitor- 1), a protein isolated from a brain library for its ability to be regulated by p53, has also been shown to contain the type I repeat of TSP- I and to provide anti-angiogenic potential to this molecule (Nishimori, H., et al., Oncogene

15:2145-2150 (1997)). Nevertheless, it appears that additional sequences or context are also important, since other proteins containing the type I repeats appear not to have clear or more established anti-anglogenic properties such as: properdin, F-spondin, and other members of the complement family.

Because of the presence of TSP-repeats in METHI and MEETH2, along with their anti-angiogenic properties, these proteins were originally considered members ofthe TSP superfamily. Nevertheless, they have no additional homology to other TSPs, and in fact, the similarity to TSPI and TSP2 is restricted to the type I repeats. Furthermore, the proteins also have strong sequence and structural homology to members of the ADAM family. These features led Kuno and colleagues to name ADAMTS to the mouse homolog of METHI (Kuno, K., et aL, J BioL Chem. 272:556-562 (1997)). The recent identification of pNPI and its striking sequence homology to the proteins here described, prompt all these three proteins to be grouped in a subfamily named metallospondins. At this point, it is not clear whether pNIP has anti-angiogenic properties or whether METHI and/or METI-12 participate in the cleavage of the amino terminal pro-peptide of a I (I) procollagen.

Example 2: Northern and Southern blot analysis Total RNA was purified from cells by guanidinium-isothiocyanate extraction, as previously described (Chomczynski, P. & Sacchi, N., AnaL Biochem. 162:156-159 (1987)) Poly(A)+RNA was extracted using a Boehringer Mannheim (BMB, Indianapolis, IN) kit according to the manufacturer conditions.

Other poly(A)+RNA blots were purchased from Clontech (Palo Alto, CA). Pre-hybridization was performed in a solution containing: 50% formamide, 6X SSPE, IX Denhardt's solution, 0. I % SDS and I 00@tg/ml of heat denatured salmon sperm DNA for 12-18h at 420C. Hybridization with labeled cDNA probes proceeded in the same solution at 42'C for 12-18h. T SP I and METH I probes corresponded to the entire human cDNAs. METI-12 probe corresponded to a KpnI-EcoRI fragment from the human cDNA. A 1. 3 Kb Pstl fragment of the glyceraldehyde-3 - phosphate-dehydrogenase (GPDH) was used to normalize for loading and transfer efficiency. Membranes were exposed to Kodak Biomax MS film (Kodak, New Haven, CT).

For Southern blots, human genomic DNA, purchased from Promega (Madison, WI), was heated at 65'C for IO min and digested with EcoRl and Pstl overnight at 37'C. 5@ig of digested DNA was separated in a 1% agarose gel, transferred to a nytran membrane and cross-linked by ultraviolet light. cDNA probes, as well as, prehybridization and hybridization conditions were identical to thosedescribedforNorthernblots. Blotswerewashedwithhighstringency(O.2X SSCI 0.2% SDS at 500C).

The expression pattern of METHI and METH2 was examined in both adult and embryonic tissues. Northern blot analysis was performed under high- stringency conditions with blots that included poly(A)+RNA from human tissues, METHI and METH2 transcripts revealed a single band of 4.6 and 3.7Kb, respectively. Abundant METHI mRNA expression was observed in adrenal, heart, placenta, followed by skeletal muscle, thyroid and stomach. From the embryonic tissues analyzed, kidney showed the highest expression of METHI mRNA. Nevertheless, weaker expression of METHI mRNA was seen in all tissues analyzed. Distribution of METH2 mRNA was more restricted and weaker than that of METH I. The highest expression was seen in lung, both embryonic and adult. Interestingly, METHI and METH2 expression do not appear to overlap. In combination, the structural similarities and their pattern of expression suggest functional redundancy yet different transcriptional regulation. The expression levels of TSP I transcripts in the same blots were also analyzed, for purpose of comparison. TSP I mRNA highest expression was seen in the adult placenta and in all embryonic tissues analyzed. In contrast to METHI and METH2 we observed constant levels of TSPI transcript in all the other tissues examined.

The cell type distribution was also studied by Northern blot analysis of poly(A)+RNA. METHI mRNA was detectable, at low levels, in dermal fibroblasts, vascular smooth muscle, endometrial stromal cells, and in two cancer cell lines, HeLa and G63 1, an adenocarcinoma and a melanoma, respectively.

NIETH2 mRNA was detected only on SW480, a colon carcinoma cell line, but no expression was seen in any other of the cell lines or primary strains analyzed.

The possibility that groups of angiogenic and anti-angiogenic factors regulate vascular network formation in specific organs has been a frequently discussed hypothesis likely to be true, yet unproven. The expression patterns of METH I and METH2, which are clearly distinct and almost non- overlapping, were puzzling, at least with concern to overall

levels. TSPI and TSP2 also share identical structure, high level of amino acid similarity, yet their pattern of expression differs significantly (Iruela-Arispe, M.L., Dev. Dyn. 197:40-56 (1993)).

The differences are likely based on dissimilar cis-acting elements in their promoters and different regulatory mechanisms, as previously suggested.

Although the promoters for METH I and 2 have not been characterized, it is likely that they provide unique features for the regulation of each gene. Nevertheless, the possibility that one motif, the anti-angiogenic / type I repeat, with demonstrated anti-angiogenic properties is present in several proteins with different tissue specificities is appealing. Alternatively, the small differences in sequence between closely related members of the same family could possess significance that goes beyond functional redundancy. In the case of TSP I and TSP2, aside from the striking structural similarities and perhaps having functionally common anti-angiogenic properties, TSP I and TSP2 also appear to display functions of their own and not likely shared by their similar relative. This became evident with the outcome of the two knock-outs for these genes. TSP 1 null animals exhibited primarily lung disorders (Lawler, J., el aL, J Clin. Invest.

101:982-992 (1998)) and secondarily vascular abnormalities, but only under specific pathological settings or on a restricted set of organs. In contrast TSP2 knock-out mice exhibited unpredicted collagen assembly anomalies, with carry-on consequences to the skin, tendons, and bone (Kyriakides, T. R., et aL, J Cell BioL 140:419-430 (1998)). In addition, these animals also appear to have overall increase in capillary density in the dermis. It is not understood how the resemblance between the newly described members of the metallospondin family translate functionally. Clearly, pNIP has been shown to display active proteolytic activity by cleaving the N-terminus oftype I procollagen (Colidge, A., et al., Proc.

Nat/. Acad. Sci. USA 94:2374-2379 (1997)).

A second region of functional interest corresponds to the disintegrin domain. This domain has been more fully characterized in related members of the snake venom metalloproteases that have been shown to bind to UIIbP3 and inhibit platelet interaction blocking coagulation (Pfaff, M., et al., Cell Adhes Commun.

2:491-501 (1994); Usami, Y., et al., Biochem. Biophys. Res. Commun. 201:33 1 - 339(1994)). Thedisintegrinmotifconsistsofathirteento frequently contain an RGD or a negatively charged residue at the position of the aspartic acid. The RGD, or equivalent, binds to integrins and serve as antagonist or signaling ligands (Wolsberg, T.G. & White, J.M., Developmental Biology 180:389-401 (1996)). METH2, but not METHI, has an RGD sequence located amino-terminal to the disintegrin domain. In addition, both molecules present relatively high, but not perfect, degree of conservation of cysteines within the disintegrin motif This appears to display an important role in the tertiary structure of this region and its ability to interact with integrins. In addition, some of these domains have been shown to act as functional adhesion molecules, particularly those with transmembrane regions (Wolsberg, T.G. & White, J.M., Developmental Biology 180:3 89-401 (1996)). It is unlikely that this will be the case for METHI and METH2, since both these proteins appear to be secreted.

Example 3: Expression andpuriflication of recombinantproteins Recombinant constructs for expression of truncated fusion proteins were as follows: (1) pRSET-NffiTHI-Type 1: METHI nt 1605-1839 (from the start codon) was amplified by polymerase chain reaction using the following primers.

5'-GCA TTT TGG ATC CGC CTT TTC ATG-3 / (SEQ ID NO: 78) and 5'-GTT GTGTGCTGCAGATTGTTCC-3' (SEQIDNO:79). Theamplifiedfragnient was then subcloned into the BamHI and Pstl sites of the pRSET vector- (2) pGEX-METH I -TSP was generated by ligating the BamHI-EcoRl fragment from the pRSET-NIETH I -TSP into the Smal site of the pGEX-5X vector (Pharmacia Biotech Inc., Piscataway, NJ) by blunt-end ligation; (3) pGEX- I -O-NMTH2: the fragment nt 838-1818 of METH2 cDNA (from the start codon) was ligated into Bamffl-Ecoltl sites ofpGEM-2TK. The METH2 fragment was amplified by PCR using the following primers: 5'-GAAAAATGGGGATCCGAGGTG-3'(SEQ ID NO: 80) and 5'-GCAGGAGAATTCCGTCCATG-3'(SEQ ID NO:8 1) to generate BamHl and EcoRl restriction sites; (4) pGEX-NffiTH2-TSP: a 0.5Kb Xmal- EcoRI fragment isolated from pGEX- 1. 0-METH2 was subcloned into the Xn?aI and EcoRI sites of pGEX-2TK vector. All constructs were sequenced to verify sequence fidelity and correct open reading frame.

The recombinant proteins were named 6H-METHI, the recombinant protein expressed with the plasmid pRSET-NIETHI-TSP, GST-METHI, the protein expressed with the plasmid pGEX-N4ETH I -TSP and GST-METH2, the protein expressed with the plasmid pGEX-METH2-TSP.

Expression plasmids were transformed into BL21:DE3 E. co/i strain (Stratagene Cloning Systems, La Jolla, CA) and fusion proteins were induced following manufacturer recommendations. Briefly, induced bacteria pellets were resuspended in PBS and sonicated on ice for l min. The suspension was, subsequently, incubated at RT for 20min in the presence of I% triton X- I 00 and centrifuged at 4'C. Histidine tagged fusion proteins were then purified on Ni-NTA beads (Qiagen, Chatsworth, CA) by incubating 20nil of supernatant with I ml of beads (50% slurry) for 2h at 4'C. The suspension was transferred into a column and washed with I 0 columns volume of PB S containing I OMM imidazole, followed by 5OmM imidazole and finally I OOmM imidazole. The protein was eluted with 5OOmM imidazole in PBS. Fractions containing the recombinant protein were dialyzed against phenol-red free DNIEM. Samples were centrifuged for 30min at 4'C, part of the protein was not soluble and was lost during centrifugation. The supernatant was stored at -70'C and used for proliferation, cornea pocket and chorioallantoic membrane (CAM) assays.

For purification of GST-fusion proteins, the extract was cleared by centrifugation and applied to a GST-affinity column (Pharmacia). The column was washed with PBS-l% triton X-100 in the presence of O.ImM reduced glutathione and, subsequently, with the same buffer in the presence of 0.5mM reduced glutathione. Fusion proteins were eluted with I OmM reduced glutathione in 50mM Tris-HCI, pH 7 Fractions containing the protein were dialyzed against DNffiM, stored at -70'C and used for proliferation, cornea pocket and chorloallantoic membrane (CAM) assays.

Integrity and purity of recombinant proteins was analyzed in 12.5% or 15% acrylamide gels stained with Coomassie blue.

A recombinant GST fusion protein containing the first two type I repeats of TSP was also dialyzed against DMEM before used in functional assays. Intact TSP I was purified from platelets as previously described (Roberts, D.D., et aL, J. Tissue Cult. Methods 16:217-222 (1994)).

To test the hypothesis that NffiTHI and METH2 TSP domains could function as regulators of angiogenesis recombinant fusion proteins were generated in bacteria. The constructs included the first TSP domain of METHI or METH2.

This domain is the most conserved, 52% amino acid similarity with the second type I repeat of TSPI, (this domain contains a putative binding site for CD36).

All recombinant proteins were isolated under native conditions to preserve their secondarystructureasmuchaspossible. 6H-WTHI andGST-NfETHI contained the first TSP-like domain of NfETHI fused to a histidine tag or a GST, respectively. NEETHI recombinant protein was made with two different tags because of purification and structural advantages. The differences in size are due to the size of the tag, 6KDa the histidine and 27KDa the GST. GST-METH2 contained the first TSP domain of NIIETH2 also fused to a GST. A fragment corresponding to the last two type I repeats of TSPI, also fused to a GST, and intact TSPI purified from platelets were used as positive controls. In addition, GST alone was included in all experiments as negative control.

Example 4: TSP domains in METHI andMETH2 disrupt angiogenesis in vivo Cornea pocket assay Swiss Webster females and males, were purchased from Charles River (Boston, MA) and used between 8- 1 0 weeks-old for implantation of the pellets.

Cornea pockets were performed as described by Kenyon and colleagues (Kenyon, B.M., et al., Invest. Ophthalmot, Vis. Sci. 37:1625-1632 (1996)) with few modifications. Briefly, a solution of 10@tg of recombinant bFGF plus 5 mg of sucralfate were mixed with 10@tl of Hydron (200mg/ml in ethanol; New Brunswick, NJ) and the recombinant protein of interest (2[tg). The suspension was then smeared onto a sterile nylon mesh square (pore size 500[im; Tetko Inc., Briarcliff Manor, NY) and allowed to dry for 30min. The fibers of the mesh were pulled to produce pellets of 50OVm' that were stored at -20'C. Uniformly sized pellets were selected under a microscope and used for the assays.

Mice were anesthetized with Avertin. An incision was made in the cornea using a Nikon SMZ-U dissecting microscope with the aid of a surgical blade. A single pellet was implanted into the pocket. Five days after pellet implantation, corneal angiogenesis was evaluated and photographed.

CAM assay Chorioallantoic membrane assays were performed on Leghorn chicken embryos (SPAFAS, MA) at 12-14 days of embryonic development. Matrigel (750pg/ml), VEGF (250ng/mesh) and the protein or peptide to be tested were mixed, placed onto nylon meshes (pore size 250@tm; Tetko Inc.) and incubated sequentially at 37'C for 30min and at 4'C for 2h to induce polymerization. A positive (matrigel and VEGF) and a negative (VEGF alone) control were also prepared for each CAM. Polymerized meshes were placed onto the third outer region of the CAM and incubated for 24h. To visualize vessels, 400@11 of fluorescein isothlocyanate dextran (10mg/ml, SIGMA) was injected in the chick bloodstream. A-fter5-10minincubation,thechickwastopicallyfixedwith3.7% formaldehyde for 5min. The meshes were then dissected and mounted onto slides.

Fluorescence intensity was analyzed with a computer-assisted image program (NIH Image 1. 5 9).

Peptides used on these assays were synthesized by Chiron (Raleigh, NC).

Sequence corresponded to amino acids: P-TSP 1, 43 0 P-METHI, 549-563 P-METH2, 529 The evaluation of anglogenic or anti-angiogenic responses relies heavily on the sensitivity and specificity of the assays used to assess the response. To evaluate the anti-angiogenic activity of these fragments in vivo, two popular and well-accepted angiogenesis assays were used: the corneal pocket and the chorioallantoic membrane. The visibility, accessibility, and avascularity of the cornea are highly advantageous and facilitate the visualization of the neovascular response and the topical application of the test substances. A known amount of angiogenesis factor(s) is implanted, as a pellet, in a pocket made in the cornea eye.

To test an angiogenesis inhibitor, the molecule is implanted with the stimulator in the same pellet, and the response is compared to the stimulator alone.

In these experiments, bFGF was used as the vascularization stimulator.

Pellets containing the recombinant protein were implanted in mouse corneas and their ability to inhibit the bFGF-induced angiogenic response was compared to that of controls. When a bFGF pellet containing GST was implanted new capillary vessels grew from the cornea limbus, across the cornea and into the pellet within 5 days. In contrast, addition of GST-METHI or GST-METH2 to the bFGF pellets completely abolished blood vessel growth. Table 4 contains a summary of the results obtained from 41 assays performed. Intact TSP I purified from platelets and GST-TSP I were used as positive controls. All assays were performed at identical concentrations, suggesting that METHI and N1ETH2 have similar potency to that of TSP I in the inhibition of angiogenesis. In addition, when half of the standard concentration was used, a weak, however noticeable response was seen, indicating a dose-dependent effect.

Table 4.

Activity of METHI and METH2 recombinant proteins in the corneal pocket assay bFGF Pellets T-Vascularized corneas/Total corneas Vehicle 5/5 TSPI 0/5 GST 11/11 GST-TSPI-TI 1/4 GST-METHI-TSP 0/8 GST-METH2-TSP 0/8 In the CAM assay, the anglogenic response is analyzed by measuring the number of vessels that grow within a matrix polymer containing the anglogenic gro-xth factor. To determine whether recombinant NIETH I and NMTH2 proteins inhibited neovascularization in the CAM assay induced by VEGF, a matrigel polymer containing VEGF and the recombinant protein were implanted in the CAM. Quantitative analysis of the experiments, which included three different polymers per treatment are shown in Figure 6A. Matrigels polymers containing VEGF plus 5,ug of GST-METHI or GST-METH2 caused greater than 80% inhibition in blood vessel growth. A similar potency was found using the GST recombinant protein derived from the type I repeats of TSP I. Furthermore, the anti-angiogenic effect of the TSP domains in METHI and METH2 was dose- dependent with a complete inhibition of blood vessel growth when 15 ug/ml of protein was used (Figure 6C and D). GST alone, at identical concentrations, had no significant effect on VEGF-stimulated angiogenesis.

Synthetic peptides from the second or the third type I repeats of human TSP I can mimic that anti-angiogenic effects

of the intact TSP I (Tolsma, S. S., el al., J. Cell. BioL 122:497-511 (1993)). In fact, a 19-residue polypeptide was shown to be sufficient to block in vivo neovascularization in the rat cornea and to inhibit the bFGF-induced migration of cultured endothelial cells (Vogel, T., et aL, J Cell. Biochem. 53:74-84 (1993)- Tolsma, S. S., et aL, J Cell. BW 122:497-51 1 (1993)). To test whether the same was true for the NMTHI and METH2 TSP domains, peptides derived from the same region were synthesized and their anti- angiogenic activity was evaluated in the CAM assay. The results are shown in Figure 613. Peptides derived from both the TSP domain of METHI and N4ETH2 blocked VEGF-induced angiogenesis similarly to that of TSPL In contrast, scramble peptides had no significant effects.

Example 5:,Prol@feration assays Human dermal endothelial cells (HDEC) were isolated and grown on VitrogenTM coated petri-dishes in EBM (Clonetics, San Diego, CA) supplemented with 15% fetal calf serum, 25@tg/ml cAMP, and 1@tg/nil of hydrocortisone acetate and were used from passages 3 to 6. Cells were made quiescent by incubation of confluent monolayers with phenol red-free EBM containing 0.2% BSAfor48h. Humandermalfibroblastswerelsolatedfromne by enzymatic dissociation. Both fibroblasts and smooth muscle cells were maintained in DMEM supplemented with I 0% fetal calf serum. Human mammary epithelial cells (FEMEC) were purchased from Clonetics and maintained in the recommended media (mammary epithelial growth media, MEGM).

Quiescent human dermal endothelial cells, between passage 3 and 6, were plated on VitrogenTM coated 24-well plates in EBM supplemented with 0.2% BSA, 0. I% fetal calf serum and I ng/nil of bFGF in the presence or absence of the recombinant protein and incubated at 5% C02 at 37'C for 48h. For vascular smooth muscle (VSM) and fibroblast proliferation assays, cells were incubated under the same conditions but using DMEM instead of EBM. Human mammary of [3 epithelial cells were incubated on their growth media. A pulse H]-Thymidine (I [tCi/[tl) was added during the last 4h prior harvesting. Cells were washed and fixed in 10% TCA. Incorporation of ['H]-thymidine was determined by scintillation counting, as previously described (Iruela-Arispe, M.L. & Sage, E.H., J. Cell. Biochem. 52:414 (1993)).

Statistical analysiswere doneusing In-Stat software (GraphPad Software) for Macintosh. Assuming normal distributions, data were analyzed by one-way ANOVA, followed by either T-test Dunnett test for comparisons between groups, or student-Newman-Kleus test for multiple comparisons between groups.

To gain insight into the mechanism by which METH I and METH2 inhibit neovascularization, the direct effect of the purified recombinant fusion proteins on endothelial cell proliferation was tested. Serum-starved endothelial cells were plated into growth medium containing bFGF and FCS. Recombinant proteins (3[tg/ml)wereaddedatthesametimeofplating. 40GST-WTHl), 456H- GST) or 36% (GST-METH2) inhibition was observed, in contrast to a non-significant effect when GST alone was added. The recombinant protein from the type I repeats of TSP I had similar inhibitory effects. (Figure 7A). Furthermore, suppression of proliferation mediated by METHI or METH2 were dose-dependent, as shown in Figure 7E. The inhibition was observed as early as one day after treatment and the inhibitory effect was not toxic and reversible since the removal of the recombinant protein and subsequent addition of growth factor alone led to the resumption of endothelial cell proliferation.

The cell specificity of the anti-proliferative effects for METHI and METH2 on the endothelium was evaluated by additional proliferation assays on a variety of non-endothelial cells. No significant inhibition of proliferation was seen on fibroblasts or smooth muscle cell cultures. In contrast, a non significant, but reproducible stimulation of proliferation for these two cell types could be observed. This result rules out the presence of any potential nonspecific inhibitor of cell growth in the recombinant protein preparations. On mammary epithelial cell, however, METH I and METH2 inhibited cell proliferation to the same degree as to endothelial cells. Interestingly, TSP I also suppresses mammary epithelial cell proliferation both in vitro and in a transgenic model.

The possibility that METHI and METH2 might act as disintegrins is consistent with their anti-angiogenic properties. Clearly blockade of UvP3 and P I integrins with antibodies has been shown to inhibit neovascularization both during development and in tumors (Brooks, P.C., et aL, Cell 85:683 -693 (1996); Brooks, P.C., et aL, Cell 92:391-400 (1998); Senger, DR., et al., Proc. Naff Acad. Sci.

USA 94:13612-13617 (1997)). Integrins are essential for the mediation of both proliferative and migratory signals (Schwartz, M.A. & Ingber, D.E., AlloL BioL Cell 5:389-393 (1994)), therefore interference with those signals can be highly deleterious to the angiogenic process. The angiogenic functional assays were performed with recombinant

protein containing only the type I repeats in METH I and METH2.

1.5 The mechanism of action of METHI and METH2 with regards to their angio-inhibitory activity is not known. To date we have evidence that these proteins are secreted and bind to endothelial cells. Further investigations are guided towards the identification ofreceptors and signal transduction mechanisms.

A likely hypothesis resulting from the lessons learned from TSP I is that both METHI and METH2 bind to CD36. Recently, this scavenger receptor has been implicated in the mediation of signals by which TSP- I exert its anti-angiogenic effects (Dawson, D.W., et aL, J Cell. Biot 138 717 (1997)). Both the CSVTCG (SEQ ID NO:83) (Asch, A.S., et aL, Nature 262:1436-1439 (1993))- Catimel, B., et aL, Biochein. J 284:231-236 (1992)) and the GCQXR (SEQ ID NO:84) sequences have been proposed as primary binding motifs to CD36 (Dawson, D.W., et aL, J Cell. BioL 138:707-717 (1997)). METH I and METI-12 have almost entire conservation in both these regions. A complementary and also likely occurrence is binding of METHI and METI-12 to bFGF. Binding to heparin andbFGFhasbeenproposedaspartoftheanti-angiogenicactivityofTSPI (Guo, N., et aL, J Peptide Res. 49 (1997)). This property appears to be mediated through the WSXWS (SEQ ID NO:82) motif, also conserved in METHI and METH2. Future efforts will focus on the signals implicated in the anti-angiogenic properties mediated by these novel proteins and on their potential as proteases of the extracellular milieu.

Example 6: Isolation qf the METHI or METH2 cDNA Clone From the Deposited Sample Two approaches can be used to isolate METH] or METH2 from the deposited sample. First, the deposited clone is transformed into a suitable host (such as XL- I Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. A single colony is then used to generate DNA using nucleic acid isolation techniques well known to those skilled in the art. (e.g., Sambrook et al., Molecular Cloning- A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press.) Alternatively, two primers of 17-20 nucleotides derived from both ends of the SEQ ID NO A or SEQ ID NO: 3 (i.e., within the region of SEQ ID NO: I or SEQ ID NO:3 bounded by the 5'NT and the TNT of the clone) are synthesized and used to amplify the METHI or METH2 cDNA using the deposited cDNA plasmids as templates. The polyinerase chain reaction is carried out under routine conditions, for instance, in 25 @tl of reaction mixture with 0.5 @tg of the above cDNA template. A convenient reaction mixture is 1 - 5 - 5 MM MgC'2, 0 - 0 1 % (W/V) gelatin, 20 uM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94 degree C for I min; annealing at 55 degree C for I min; elongation at 72 degree C for I min) are performed with a Perkin-Elmer Cetus automated thermal cycler.

The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subdoning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of the METH I or NIETH2 gene which may not be present in the deposited clones. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5'and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full- length transcript. (Fromont-Racine el al., Nucleic Acids Res. 2] (7):1683-1684 to (1993).) Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts, A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the METH I or MEETH2 gene of interest is used to PCR amplify the 5'portion of the METHI or NIETH2 full-length gene.

This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5'end sequence belongs to the METHI or METH2 gene.

Example 7. Bacterial Expression of METHI or METH2 A METHI or METI-12 polynucleotide encoding a METHI or METI-12 polypeptide invention is amplified using PCR oligonucleotide primers corresponding to the 5'and Yends of the DNA sequence, as outlined in Example 5, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and Xbal, at the 5' end of the primers in order to clone the amplified product into the expression vector.

For example, BamHI and Xbal correspond to the restriction enzyme sites on the bacterial expression vector pQE (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp'), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/0), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites. The pQE-9 vector is digested with BamI-H and Xbal and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS.

The ligation mixture is then used to transform the E. coli strain MI 5/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan @. Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (I 00 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1: I 00 to 1:250. Thecellsaregrowntoanopticaldensity6OO(O.D.'")ofbetweeno.4and IPTG (Isopropyl-B-D-thlogalacto pyranoside) is then added to a final concentration of I mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 600OXg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HO by stirring for 3-4 hours at 4 degree C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., supra). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QlAexpressionist (I 99 5) QIAGEN, Inc., supra).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCI, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCI, pH 8, then washed with 10 volumes of 6 M guanidine-HCI pH 6, and finally the polypeptide is eluted with 6 M guanidine-110, pH 5.

The purified METHI or METH2 protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the METH I or METH2 protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-IM urea gradient in 500 MM NaC1, 20% glycerol, 20 mM Tris/HCI pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM immidazole.

Immidazole is removed by a final dialyzing step against PBS or 50 MM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified METH I or METH2 protein is stored at 4'C or frozen at -80'C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a METH I or METH2 polynucleotide, called pHE4a. (ATCC Accession Number 209645, deposited February 25, 1998.) This vector contains.

1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coh origin of replication, 3) a T5 phage

promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lac1q).

The origin of replication (oriC) is derived from pUC 1 9 (LTI, Gaithersburg, MD).

The promoter sequence and operator sequences are made synthetically, DNA can be inserted into the pHEa by restricting the vector with Ndel and Xbal, BamHl, Xhol, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 3 1 0 base pairs).

The DNA insert is generated according to the PCR protocol described in Example 5, using PCR primers having restriction sites for Ndel (5' primer) and Xbal, BamH1,XhoI,orAsp718(3'primer). ThePCRinsertisgelpurifiedandrestricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

Example 8: Purification ofMETHI orMETH2 Polypeptidefrom an Inclusion Body The following alternative method can be used to purify WTHI or METH2 polypeptide expressed in E coh when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-100C.

Upon completion of the production phase of the E. coh fermentation, the cell culture is cooled to 4-10 degree C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing I 00 mM Tris, 5 0 mM EDTA, pH 7 The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfuidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0. 5 M NaCI, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0. 5M NaCl, I 00 mM Tris, 5 0 mM EDTA, pH 7 The resulting washed inclusion bodies are solubilized with 1. 5 M guanidine hydrochloride (GuHCI) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4 degree C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4 degree C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0. 16 um membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive Biosystems). The column is washed with 40 MM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, I 000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the NMTH I or METH2 polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosysterns) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH Both columns are washed with 40 mM sodium acetate, pH 6.0@ 200 MM NaCI. The CM-20 column is then eluted using a I 0 column volume linear gradient ranging from 0. 2 M NaCI, 5 0 mM sodium acetate, pH 6. 0 to 1. 0 M NaCI, 5 0 mM sodium acetate, pH 6 Fractions are collected under constant AM, monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant NIETH I or METH2 polypeptide should exhibit greater than 95 urityaftertheaboverefoldingandpurificati. Nomajorcontaminant

bandsshouldbeobservedfromCoomassiebluestDS-PAGEGELWHEN 5 UG OF PURIFIED PROTEIN IS LOADED. THE PURIFIED METH I OR METH2 PROTEIN CAN ALSO BE TESTED FOR ENDOTOXIN/LPS CONTAMINATION, AND TYPICALLY THE LPS CONTENT IS LESS THAN 0. I NG/ML ACCORDING TO LAL ASSAYS.

Example 9: Cloning and Expression ofMETHI or METH2 in a Baculovirus Expression System In this example, the plasmid shuttle vector pA2 is used to insert METH I or METH2 polynucleotide into a baculovirus to express METHI or METH2.

This expressionvector contains the strongpolyhedrinpromoter oftheAutographa califomica nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40") is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from K coh under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned NMTHI or METH2 polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIMI, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., Virology 170:31-39 (1989).

Specifically, the METHI or METI-12 cDNA sequence contained in the deposited clone, including the AUG initiation codon and any naturally associated rib i leader sequence, is amplified using the PCR protocol desc i ed in Example 5. if the naturally occurring signal sequence is used to produce the secreted protein, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose get using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. E. coli HB I 0 1 or other suitable E coli hosts such as XL- I Blue (Stratagene Cloning Systems, La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five ug of a plasmid containing the polynucleotide is co-transfected with 1.0 ug of a commercially available linearized baculovirus DNA ("BaculoGold' baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987).

One ug of BaculoGold'virus DNA and 5 ug of the plasmid are mixed in a sterile well of a microtiter plate containing 50 ul of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, NED). Afterwards, 10 ul Lipofectin plus 90 ul Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 171 1) seeded in a 3 5 mm tissue culture plate with I ml Grace's medium without serum. The plate is then incubated for 5 hours at 27 degrees C.

The transfection solution is then removed'from the plate and I ml of Grace's insect medium supplemented with IO% fetal calf serum is added. Cultivation is then continued at 27 degrees C for four days.

to After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, supra. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-1 0.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 ul of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 3 5 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4 degree C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with I 0% heat-inactivated FB S. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOP) of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 11 medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, NM). After 42 hours, 5 UCi of 15S-methionine and 5 UCi 15 S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced METH I or METH2 protein.

Example IO: Expression of METHI or METH2 in Mammalian Cells METH I or METH2 polypeptide can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals 0 required for the termination of transcription and polyadenylation of the transcript.

Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharinacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2DHFR (ATCC 37146), pBC12MI (ATCC 67109), pCMVSport 2.0, and pCMVSport 3 Mammalian host cells that could be used include, human Hela, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QCI-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, METH I or METH2 polypeptide can be expressed in stable cell lines containing the METHI or METH2 polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as DBFR, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected METH I or METH2 gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., el al., J Biol.

Chem. 253:13 5 7-13 70 (1978); Hamlin, J. L. and Ma, C., Biochem. el Biophys, Acia 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64- 68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J 22 7:277-279 (199 1); Bebbington et al., Bio/Technology 10: 169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinesehamsterovary(CHO)andNSOcellsareoftenusedforthe production of proteins.

Derivatives of the plasmid pSV2-DHFR (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No.209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen el al., Molecular and Cellular Biology, 43 8-447 (March, 1985)) plus a fragment of the

CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHl, Xbal and Asp718, facilitate the cloning of METHI or METH2. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

If a naturally occurring signal sequence is used to produce a secreted protein, the vector does not need a second signal peptide. Alternatively, if a naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence in an effort to secrete the protein from the cell. (See, e.g., WO 96/34891.) The amplified fragment is then digested with the appropriate restriction enzyme and purified on a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. E. coli HB I 0 1 or XL- I Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 or pC4 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five @ig of the expression plasmid pC6 or pC4 is cotransfected with 0.5ugoftheplasmidpSVneousinglipofectin(Felgneretal.,supt-a). Theplasmid pSV2-neo contains a dominant selectable marker, the neo gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus N/IEM supplemented with I mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus I mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 6M, 100 rM, 200 nM, 400 rM, 800 rA4).

Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (I UM, 2 uM, 5 uM, IO mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 uM. Expression of METH I or N1ETH2 is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase IIPLC analysis.

Example 11: Construction of N-Terminal andlor C-Terminal Deletion Mutants The following general approach may be used to clone a N-terminal or C- terminal deletion METHI or METH2 deletion mutant. Generally, two oligonucleotide primers of about 15-25 nucleotides are derived from the desired 5' and 3' positions of a polynucleotide of SEQ ID NO: I or SEQ ID NO: 3. The 5'and Ypositions of the primers are determined based on the desired METHI or METH2 polynucleotide fragment. An initiation and stop codon are added to the 5' and 3' primers respectively, if necessary, to express the METHI or METH2 polypeptidefragmentencodedbythepolynucle. PreferredMETHI or NIETH2 polynucleotide fragments are those encoding the N-terminal and C- terminal deletion mutants disclosed above in the "Polynucleotide and Polypeptide Fragments" section of the Specification.

Additional nucleotides containing restriction sites to facilitate cloning of the METH I or METH2 polynucleotide fragment in a desired vector may also be added to the 5'and Yprimer sequences. The NIIETHI or NIIETH2 polynucleotide fragment is amplified from genomic DNA or from the deposited cDNA clone using the appropriate PCR oligonucleotide primers and conditions discussed herein or known in the art. The METHI or METH2 polypeptide fragments encoded by the NIEETHI or METH2 polynucleotide fragments of the present invention may be expressed and purified in the same general manner as the full length polypeptides, although routine modifications may be necessary due to the differences in chemical and physical properties between a particular fragment and full length polypeptide.

As a means of exemplifying but not limiting the present invention, the polynucleotide encoding the METHI polypeptide fragment D-40 to S-950 or the METH2 polypeptide fragment L-20 to L-890 is amplified and cloned as follows.

A 5' primer is generated comprising a restriction enzyme site followed by an initiation codon in frame with the polynucleotide sequence encoding the N-terminal portion of the polypeptide fragment beginning with D-40 or L-20, respectively. A complementary 3' primer is generated comprising a restriction enzyme site followed by a stop codon in frame with the polynucleotide sequence encoding C-terminal portion of the METHI or NIETH2 polypeptide fragment ending with S-950 or L-890, respectively.

The amplified polynucleotide fragment and the expression vector are digested with restriction enzymes which recognize the sites in the primers. The digested polynucleotides are then ligated together. The NMTHI or NETH2 polynucleotide fragment is inserted into the restricted expression vector, preferably in a manner which places the NIETH I or NIIETH2 polypeptide fragment coding region downstream from the promoter. The ligation mixture is transformed into competent E coh cells using standard procedures and as described in the Examples herein. Plasmid DNA is isolated from resistant colonies and the identity of the cloned DNA confirmed by restriction analysis, PCR and DNA sequencing.

Example 12: Protein Fusions of METHI or METH2 NIETHI or METH2 polypeptides are preferably fused to other proteins.

These fusion proteins can be used for a variety of applications. For example, fusion of NIETH I or METH2 polypeptides to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See Example 7; see also EP A 394,827, Traunecker, et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG- 1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to METHI or METH2 polypeptides can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function.

Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 7.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5'and Yends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3'BamHI site should be destroyed. Next, the vector containing the human Fc portion is re- restricted with BamHl, linearizing the vector, and METHI or METH2 polynucleotide, isolated by the PCR protocol described in Example 5, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a ftision protein will not be produced.

If the naturally occurring signal sequence is used to produce the secreted protein, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.) to Human IgG Fc region.

GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATG
GTGCCCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTC
CCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACTCC
CACATGCGTGGTGGTGGACGTAAGCCACGAAGACCCTGAG
TTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCA
AGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGT
CCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAG
TGCAAGGTCTCCAACAAAGCCCTCCCAACCCCCATCGAGA
TCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTA
GCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGC
TGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGTGG
AGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCC
GCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTC
ACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC
GCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC
TCTCCGGGTAAATGAGTGCGACGGCCGCGACTCTAGAGGA (SEQ ID NO:85) Example 13: Production of an Antibody The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) For example, cells expressing METH I or METI-12 is administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method, a preparation of METHI or METI-12 protein is

prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

In the most preferred method, the antibodies of the present invention are I 0 monoclonal antibodies (or protein binding fragments thereof). Such monoclonal antibodies can be prepared using hybridoma technology. (Kohler et aL, Nature 256:495 (1975); Kohler et aL, Eur. J ImmunoL 6:511 (1976)- Kohler et aL, Eur.

J InnnunoL 6:292 (1976)- Hammerling et aL, in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981).) In general, such procedures involve immunizing an animal (preferably a mouse) with METH I or METI-12 polypeptide or, more preferably, with a secreted METHI or METH2 polypeptide-expressing cell. Such cells may be cultured in any suitable tissue culture niediurn- however, it is preferable to culture cells in Earle's modified Eagle's medium supplemented with IO% fetal bovine serum (inactivated at about 56 degree Q, and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 ug/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention- however, it is preferable to employ the parent myeloma cell line (SP20), available from the ATCC- After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilutionasdescribedbyWandse/aL (Gastroenterology8O:225-232(1981).) The hybridoma cells obtained through such a selection are then assayed to identif @ clones which secrete antibodies capable of binding the METHI or N/fETH2 polypeptide.

Alternatively, additional antibodies capable of binding to METHI or NIETH2 polypeptide can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoina cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the NIETHI or N1ETH2 protein-specific antibody can be blocked by METH I or M1ETH2. Such antibodies comprise anti-idiotypic antibodies to the METH I or WTH2 protein- specific antibody and can be used to immunize an animal to induce formation of further METHI or METH2 protein-specific antibodies.

It will be appreciated that Fab and F(ab')2 and other fragments of the antibodies of the present invention may be used according to the methods disclosed herein. Such fragments are typically produced by proteolytic cleavage, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). Alternatively, secreted METH I or METH2 protein-binding fragments can be produced through the application of recombinant DNA technology or through synthetic chemistry.

For in vivo use of antibodies in humans, it may be preferable to use "humanized" chimeric monoclonal antibodies. Such antibodies can be produced using genetic constructs derived from hybridonia cells producing the monoclonal antibodies described above. Methods for producing chimeric antibodies are known in the art. (See, for review, Morrison, Science 229:1202 (1985); Oi el al., BioTechniques 4:214 (1986); Cabilly et aL, U. S. Patent No. 4,816,567; Taniguchi el al., EP 171496; Morrison el al., EP 173494; Neuberger el al., WO 8601533; Robinson et aL, WO 8702671; Boulianne el aL, Nature 312:643 (1984); Neuberger el aL, Nature 314:268 (1985).) Example 14: Production Of METHI or METH2 Protein For High- Throughput Screening Assays The following protocol produces a supernatant containing METHI or METH2 polypeptide to be tested. This supernatant can then be used in the Screening Assays described in Examples 16 First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution (Img/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with Iml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at 2 x I O' cells/well in. 5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/IxPenstrep(17-602EBlowhittaker). Letthecellsgrowovernight.

Thenextday,mixtogetherinasterilesolutionbasin:300ulLipofectamine (18324-012 Gibco/BRL) and 5ml Optimem 1 (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 10- 1 2, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem 1 mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with Inil PBS.

Person A then aspirates off PB S rinse, and person B, using al 2-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem 1 complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degree C for 6 hours.

While cells are incubating, prepare appropriate media, either I %BSA in DMEM with I x penstrep, or HGS CHO-5 media (I 16.6 mg/L of CaCl2 (anhyd); 0.00130 mg/L CUS04-5H20; 0.050 mg/L of Fe(NO3)3-9H20; 0.417 mg/L of - 48.84 nigil, of MgSO4- - 31 1. 80 mg/L of Kcl; 28.64 mg/t, of MgC'2) FeS04 120, 1 6995.50 mg/L of NaCl- 2400.0 mg/L of NaHC03; 62.50 mg/L of NaH2PO4-H20; 71.02 mg/L of Na2HPOO.4320 mg/L of ZnSO4-7H20;.002 mg/L of Arachidonic Acid; 1.022 mg/L of Cholesterol- .070 mg/L of DL-alpha-Tocopherol-Acetate- 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; I 00 mg/L of Pluronic F-68; 0. 0 1 0 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine-H20; 6.65 mg/ml - 31.29 mg/ml of L- of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H20, Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid- 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52,48 mg/ml of L-Histidine-HCL-H20; 106.97 mg/ml of L-Isoleucine- 11 1.45 mg/ml of L-Leucine- 16').75 mg/ml of L-Lysine HCL 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalainine; 40.0 mg/ml of L- Proline; 26.25 mg/ml of L-Serine; 10 1. 05 mg/ml of L-Threonine- 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tryrosine-2Na-2H20; and 99.65 mg/ml of L- Valine; 0. 003 5 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; I 1. 78 mg/L of Choline Chloride- 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol- 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL- 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; 0.680 mg/L of Vitamin B,2; 25 mM of HEPES Buffer- 2.39 mg/L of I Na Hypoxanthme; 0. 105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine- 2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 2OuM of Ethanolamine; 0. 122 mg/L of Ferric Citrate,- 41.70 mg/L of Methyl-B- Cyclodextrin complexed with Linoleic Acid- 33.33 mg/L of Methyl-B- Cyclodextrin complexed with Oleic Acid; 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal Acetate. Adjust osmolarity to 327 mOsm) with 2mm glutamine and Ix penstrep. (BSA (81 3 Bayer) 100gm dissolved in IL DMEM for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degree C for 45 or 72 hours depending on the media used: I%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 3 00ul multichannel pipetter, aliquot 600ul in one I ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 16 It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the METHI or METH2 polypeptide directly (e.g., as a secreted protein) or by METHI or METH2 inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

Example 15: Construction qf GAS Reporter Construct One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathwaybind to gamma activation site "GAS " elements orinterferon- sensitive responsive element ("ISRE"), located in the promoter of many genes.

The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or " STATs, " There are six members of the STATs family. Statl and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class 1, cells after treatmentwithIL Stat5wasoriginallycalledmammarygrowthfac, buthas been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jakl, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem.

64 51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class I includes receptors for IL-2, IL-3, IL-4, IL-6, EL-7@ EL-9@ IL- 1 15 IL- 1 2, IL- 1 5, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL- I 0. The Class I receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proxial region encoding Trp- Ser-Xxx-Trp-Ser (SEQ ID NO:82)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks- STATs pathway can be identified.

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TATS -::::::::::GAS(.....

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d EFN fan-Lily IFN-a/B + + 1@2@3 ISRE I 0 IFN-g + + I GAS (IRF I >Lys6>IFP) II-lo + ? ? 1,3 9PI30 family IL-6 (Pleiotrophic) + + + ? 1,3 GAS (IRF I >Lys6> IFP) 11-II(Pleiotrophic) ? + ? ? 1,3 5 OriM(Pleiotrophic) ? + + ? 1@3 LfF(Pleiotrophic) ? + + ? 1@3 CNTF(Pleiotrophic) -/+ + + ? 1,3 G-CSF(Pleiotrophic) ? + ? ? 1,3 U.-12(Pleiotrophic) + + + 1@3 g-C family IL-2 (lymphocytes) - + - + 1 @3,5 GAS IL4 (lymph/myeloid) - + - + 6 GAS (IRF I IFP >>Ly6)(lgH) IL-7 (lymphocytes) - + - + 5 GAS IL-9 (lymphocytes) - + - + 5 GAS Il@- 1 3 (lympho cyte) - + ? ? 6 GAS IL-I 5 ? + ? + 5 GAS gpl4O family II,- 3 (myeloid) + - 5 GAS (IRF I >IFP>>Ly6) IL-5 (myeloid) + - 5 GAS GM-CSF (myeloid) + - 5 GAS Growth hon-none family GH ? + - 5 PRL ? + - I,3,5 GAS(B-CAS>IRF I =IFP>>Ly6) EPO ? + - 5 5 Receptor Tvrosme Kinases EGF ? + + - 1,3 GAS (IRF I) PDGF ? + + - 1@3 GAS (not IRFI) CSF-1 ? + + - 1,3 To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 16-17, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5'primer contains four tandem copies of the GAS binding site found in the IRFI promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an Xhol site. The sequence of the 5' primer is.

I 0 5':GCGCCTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAAT CCCCGAAATGATTTCCCCGAAATATCTGCCATCTCAATTA:3'(SEQ ID NO:86) The downstream primer is complementary to the SV40 promoter and is flanked with a I-End III site: 5': GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' 5 (SEQ ID NO: 87) PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with Xhol/Hind III and subcloned into BLSK2-.

(Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence.

5':CTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATT
GAAATGATTTCCCCGAAATATCTGCCATCTCAATTAGTCA
TAGTCCCGCCCCTAACTCCGCCCATCCCGCCCCTAACTCC
TCCGCCCATTCTCCGCCCCATGGCTGACTAATTTTTTTTA
GAGGCCGAGGCCGCCTCGGCCTCTGAGCTATTCCAGAAGT
GAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT:3' (SEQ ID NO:88) With this GAS promoter element linked to the SV40 promoter, a GAS: SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B- galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promotervector obtained from Clontechusing HIndIII and Xhol, effectively replacing the SV40 promoter with the amplified GAS - SV40 promoter element, to create the GAS-SEAP vector. However, this vector does I 0 not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEA-P vector using Sall and NotI, and inserted into a backbone vector containing the neomycin 1 5 resistance gene, such as pGFP- I (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 16 Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 18 and 19. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GASNF-KB, 11-2/NFAT,

or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), 1-fUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), RUVAC (aortic), or Cardiomyocyte.

Example 16: High-Throughput Screening Assay.for T-cell Activity The following protocol is used to assess T-cell activity of METHI or NIETH2 by determining whether METHI or METH2 supernatant proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 15. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), althoughMolt-3 cells(ATCCAccessionNo.CRL-1552)andMolt-4cells(ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Thl helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DNIRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to I mg/ml genticin 1 5 selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + IO% serum with I en-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI- MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells (I O' per transfection), and resuspend in OPTI-MEM to a final concentration of I O' cells/ml. Then add I ml of I x I O' cells in OPTI-MEM to T25 flask and incubate at 37 degree C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPM1 + 10% serum, I mg/ml Genticin, and 1% Pen-Strep. These cells are treated with .supernatants containing NIETH I or METH2 polypeptides or NIETH I or MET142 induced polypeptides as produced by the protocol described in Example 14.

On the day of treatment with the supernatant, the cells should be washed and resuspended in fresh RPMI + I 0% serum to a density of 500,000 cells per ml.

The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, I 00 million cells) are required.

I 0 Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding I 00, 000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each 1 5 well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0. 1, 1. 0, I 0 ng) is added to wells H9, HI 0, and HI I to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs).

3 5 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degree C until SEAP assays are performed according to Example 20. The plates containing the remaining treated cells are placed at 4 degree C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, I 00 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

Example 1 7. High- Throughput Screening Assay Identifying Myeloid Activity The following protocol is used to assess myeloid activity of WTHI or METH2 by determining whether NIETHI or METH2 proliferates and/or

differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 15. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF- 1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct I 0 produced in Example 15, a DEAE-Dextran method (Kharbanda et. aL, 1994, Cell, Growth & Differentiation 5:259-265) is used. First, harvest 2xlOe 7 U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetalbovine serum(FBS) supplementedwith 100 units/ml penicillin and I 00 mg/ml streptomycin.

Next, suspend the cells in I ml of 20 mM Tris-HCI (pH 7.4) buffer containing 0. 5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 MM NaC1, 5 mM KCI, 375 uM Na2HP04 120, 1 MM MgC12, and 675 uM CaCl2' Incubate at 37 degree C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degree C for 36 hr.

TheGAS-SEAP/TJ937 stablecellsareobtainedbygrowingthecellsi ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting lxI O' cells (this is enough for ten 96- well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of 5xl O' cells/ml. Plate 200 ul cells per well in the 96-well plate (or I x I O' cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example 14. Incubate at 3 7 degee C for 48 to 72 hr. As a positive control, I 00 Unit/ml interferon gamma can be used which is known to activate U937 cells.

Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 20.

Example 18:High-ThroughputSereeningAssayIdentifyingNeuro When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR I (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGRI is responsible for such induction.

Using the EGRI promoter linked to reporter molecules, activation of cells can be assessed by METH I or METH2.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGRI gene expression is activated during this treatment. Thus, by stably transfecting PC 12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC 12 cells by METH I or METH2 can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-I promoter sequence (-633 to +1)(Sakamoto K el aL, Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers.

5'GCGCTCGAGGGATGACAGCGATAGAACCCCGG -31 (SEQ ID NO:89) 5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-31 (SEQ ID NO:90) Using the GAS:SEAPNeo vector produced in Example 15, EGRI amplified product can then be inserted into this vector, Linearize the GAS:SEAP/Neo vector using restriction enzymes Xhol/HindIII, removing the GAS/SV40 stuffer. Restrict the EGRI amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one IO cm plate or 5 0 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

PC 1 2 cells are routinely grown in RPMI- 1 640 medium (Bio Whittaker) Gontaining I 0% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% beat-inactivated fetal bovine serum (FB S) supplemented with I 00 units/ml penicillin and I 00 ug/ml streptomycin on a precoated I 0 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by 5 scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 14. EGR-SEAP/PCI2 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re- grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS.

Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as 5xlO' cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent 0 to I x I O' cells/well). Add 5 0 ul supernatant produced by Example 14, 3 7 degree C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 20.

Example 19: High-Throughput Screening Assayfor T-cell Activity NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL- I and TNF, CD3 0 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS orthrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB I 0 regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- KB is retained in the cytoplasm with I- KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and 5 degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class I NMC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 14. Activators or inhibitors of NF-KB would be useful in treating diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KIB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:91), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an Xhol site.

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACT
GGACTTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:92) The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site.

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3'(SEQ ID NO:93) PCR amplification is performed using the SV40 promoter template present in the pB-gal-.promoter plasnild obtained from Clontech. The resulting PCR fragment is digested with Xhol and Hind III and subc1oned into BLSK2-.

(Stratagene) Sequencing with the T7 and T3 primers confirms the insert contains I 0 the following sequence.

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCG
TTCCATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCG
CTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCA
CCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCG 1 5
CTCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTT GGCCTAGGCTTTTGCAAAAAGCTT-31
(SEQ ID NO-88) Next, replacethe SV40minimalpromoterelementpresentinthepS- promoter plasmid (Clontech) with this NF-KB/SV40 fragment using Xhol and HindIll. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to crenerate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes Sall and Notl, and inserted into a vector containing neomycin resistance.

Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP- I with Sall and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 16.

Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 16. As a positive control, exogenous TNF alpha (0. 1, 1, 1 0 ng) is added to wells H9, H I 0, and H I 1, with a 5 - I 0 fol d activation typically observed.

Example2O: AssayforSEAPActivity As a reporter molecule for the assays described in Examples 16-19, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-fight Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set IO minutes later.

Readtherelativelightunitintheluminometer. SetHl2asblank,andprint the results. An increase in chemiluminescence indicates reporter activity.

Reaction Buffer Formulation.

--, - - - -------- - - - ----- - ----- --'- ....-- - ------- ---------- - ---- - -------- ------- of plates Rxn buffer diluent (ml) CSPD (ml) ....

10 60 3 11 65 3.25 12 70 3.5 13 75 3.75 14 80 4 15 85 4.25 16 90 4.5 17 95 4.75 18 100 5 19 105 5.25 20 110 5.5 21 115 5.75 22 120 6 23 125 6.25 24 130 6.5 25 135 6.75 26 140 7 27 145 7.25 0 28 150 7.5 29 155 7.75 30 160 8 3 1 165 8.25 32 170 8.5 5 33 175 8.75 34 180 9 35 185 9.25 36 190 9.5 37 195 9.75 38 200 10 39 205 10.25 40 210 10.5 41 215 10.75 42 220 1 1 43 225 11.25 44 230 11.5 45 235 11.75 46 240 12 47 245 12.25 48 250 12.5 49 255 12.75 50 260 13
Example 21: High- ThroughputScreeningAssay Identifying Changes in Small Molecule Concentration and Membrane Permeability Binding of a ligand to a receptor is known to alter Intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well. as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-3, used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a C02 incubator for 20 hours. The adherent cells are washed two times in Blotek washer with 200 1 0 ul of HB S S (Hank's Balanced Salt Solution) leaving I 00 ul of buffer after the final wash.

A stock solution of I mg/ml fluo-3 is made in IO% pluronic acid DMSO.

To load the cells with fluo-3, 50 ul of 12 ug/nil fluo-3 is added to each well. The plate is incubated at 37 degree C in a C02 incubator for 60 min. The plate is washed four times in the Biotek washer with HB SS leaving I 00 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells arere-suspendedto2-5xlO'cells/mlwithHBSSina5O-mlconicaltube. 4ulof I mg/ml fluo-3 solution in IO% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degree C water bath for 30-60 min.

The cells are washed twice with HBSS, resuspended to WO' cells/ml, and dispensed into a microplate, I 00 ul/well. The plate is centrifuged at I 000 rpm for 5 min. The plate is then washed once in Denley CellWash with 200 ul, followed by an aspiration step to I 00 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nni; and (6) Sample addition is 50 ul. Increased emission at 530 mn indicates an extracellular signaling event caused by the a molecule, either NffiTHI or M [ETH2 or a molecule induced by METH1 or METH2, which has resulted in an increase in the intracellular Ca" concentration.

Example22: High-ThroughputScreeningAssayIdentifyingTyros Activity The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor 1 0 subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by figands involves ligand-mediated receptor dinierization, resulting in transphosphorylation of the receptor subunits and 1 5 activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, Ick, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because ofthewiderange ofknownfactors capable of stimulating tyrosine kinase activity, identifying whether WTH I or WTH2 or a molecule induced by METHI or METH2 is capable of activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

Seedtarget cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford,MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer

Alamar Blosciences, Inc. (Sacramento, CA) after 48 hr.

Falcon plate covers #3071 from Becton Dickinson (Bedford,MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium, Cells are quiesced by incubation in serum-free basal medium for 24 hr.

1 5 After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 14, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X- I 00, 0. 1% SDS, 2 mM Na3VO4, 2 mM Na4P2O7 and a cocktail of protease inhibitors (# 183 6170) obtained from Boehringer Mannheim (Indianapolis, fN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4'C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0. 4 5 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degree C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSKI (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34)andPSK2(correspondingtoaminoacidsl-17ofgastrin). Bothpeptidesare substrates for a range of tyrosine kinases and are available from Boehninger Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add I Oul of 5uM Biotinylated Peptide, then I Oul ATP/M g2l (5niM I 0 ATP/5OmM MgC12), then lOul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, ImM EGTA, IOOMM MgC'2@ 5 MM MnCl2, 0. 5 mg/ml BSA), then 5ul of Sodium Vanadate(I MM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 3 0 degree C for 2 min. Initial the reaction by adding I Oul of the control enzyme 1 5 or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding IO ul of 120mm EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degree C for 20 min. This allows the streptavadin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phospotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(O.5u/ml)) to each well and incubate at 37 degree C for one hour. Wash the well as above.

Next add I 00ul of peroxidase substrate solution (B oehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

Example 23: High- Throughput Screening Assay Ident@fying Phosphorylation Activity As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 22, an assay which detects activation (pho sphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk- I and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p3 8 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any I 0 other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk- I or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0. Iml of protein G (lug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for I hr at RT. The

protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk- 1 and Erk-2 (I hr at RT) (Santa Cruz Biotechnology).

(To detect other molecules, this step can easily be modified by substituting a monoclonalantibodydetectinganyoftheabove.) Aftei 5 rinses with PBS, the plates are stored at 4 degree C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 14 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for I hr at RT, the wells are again rinsed.

As a positive control, a commercial preparation of MAP kinase (I Ong/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (I ug/ml) which specifically recognizes the phosphorylated epitope ofthe 3 0 Erk- 1 and Erk-2 kinases (I hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by METH I or METH2 or a molecule induced by METH I or METH2.

Example 24: Method of Determining Alterations in the METHI or METH2 Gene RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated I 0 from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regionsofinterestinSEQIDNO:I. SuggestedPCRconditionsconsistof35 cycles at 95 degree C for 30 seconds- 60-120 seconds at 52-58 degree C; and 60- 120 seconds at 70 degree C, using buffer solutions described in Sidransky, D. el al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5'end with T4 polynucleotide kinase, employing SequiTherm Polymerase. (Epicentre Technologies). The intron-exon borders of selected exons of NMTH I or METH2 is also determined and genomic PCR products analyzed to confirm the results.

PCR products harboring suspected mutations in WTHI or METH2 is then cloned and sequenced to validate the results of the direct sequencing.

PCR products of METHI or METH2 are cloned into T-tailed vectors as described in Holton, T.A. and Graham, M.W., Nucleic Acids Research 19:1156 (1991)andsequencedwithT7polymerase(UnitedStatesBiochemical). Affected individuals are identified by mutations in METHI or NMTH2 not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in the METHI or N/1ETH2 gene. Isolated genomic clones are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Manheim), and FISH performed as described in Johnson, Cg. et al., Methods Cell Biol. 3 5 99 (199 1). Hybridization with the labeled probe is carried out using a vast excess of human cot-I DNA for specific hybridization to the METHI or METH2 genomic locus.

Chromosomes are counterstained with 4,6-diamino phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson, Cv. et al., Genet. Anal. Tech. Appl. 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region of METHI or METH2 (hybridized by the probe) are identified as insertions, deletions, and 1 5 translocations. These METHI or METH2 alterations are used as a diagnostic marker for an associated disease.

Example 25: Method qfDetecting Abnormal Levels ofMETHI or METH2 in a Biological Sample METHI or METH2 polypeptides can be detected in a biological sample, and if an increased or decreased level of METHI or METH2 is

detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect METHI or METH2 in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies to METH I or METH2, at a final concentration of 0.2 to IO ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 13. The wells are blocked so that non-specific binding of METHI or METH2 to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing MIETH I or N/IETH2. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded NMTH I or METH2.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with delonized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MLT) or p-nitrophenyl I 0 phosphate (NPP) substrate solution to each well and incubate I hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot METHI or METH2 polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the 1 5 METH I or METH2 in the- sample using the standard curve.

Example 26: Formulating a Polypeptide The NIIETHI or METH2 composition will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the METHI or METH2 polypeptide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of METHI or METH2 administered parenterally per dose will be in the range of about lug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0. 0 1 mg/kg/day, and most preferably for humans between about 0. 0 1 and 1 mg/kg/day for the hormone. If given continuously, METHI or NIETH2 is typically administered at a dose rate of about I ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Pharmaceutical compositions containing METHI or N4ETH2 are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, 5 intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

N/IETHI or METH2 is also suitably administered by sustained-release systems. Suitable examples of sustained-release compositions include semi- permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules. Sustained-release matrices include polylactides (U.S. Pat. No, 317731919@ EP 58,481), copolymers of L-glutarnic acid and gamma-ethyl-L- glutamate (Sidman, U. el al., Biopolymers 22:547-556 (1983)), poly (2-hydroxyethyl methacrylate) (R. Langer et al., J Biomed Maier. Res. 15 - 167-277 (I 98 1), and R. Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (R.

Langer el al.) or poly-D- (+3-hydroxybutyric acid (EP 133,988). Sustained-release compositions also include liposornally entrapped METHI or METH2 polypeptides. Liposornes containing the METHI or NffiTH2 are prepared by methods known per se- DE 3,218,12 1; Epstein el al., Proc. Nail. Acad. Sci. USA 82:3688-3692 (1985); Hwang et aL, Proc. NatL Acad. Sci. USA 77:4030-4034 (1980); EP 52,322- EP 36,676; EP 88,046; EP 143,949; EP 142,641-Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324.

Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal secreted polypeptide therapy.

For parenteral administration, in one embodiment, NIETHI or METH2 is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to polypeptides.

Generally, the formulations are prepared by contacting METHI or METH2 uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non- aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non- toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins- chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol- counterions such as sodium- and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

METHI or METH2 is typically formulated in such vehicles at a concentration of about 0. I mg/ml to I 00 mg/ml, preferably I - I 0 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

METHI or METH2 used for therapeutic administration can be sterile.

Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutic polypeptide compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

METH I or METH2 polypeptides ordinarily will be stored in unit or multi- dose containers, for example, sealed ampoules or vials, as an aqueous solution or 5 as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, I O-ml vials are filled with 5 ml of sterile-filtered I% (w/v) aqueous NIETH I or NIETH2 polypeptide solution, and the resulting mixture is lyophilized.

The infusion solution is prepared by reconstituting the lyophilized METHI or METH2 polypeptide using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, METHI or METH2 may be employed in conjunction with other therapeutic compounds.

Example 27. Method of Treating Decreased Levels of METI-11 or METH2 The present invention relates to a method for treating an individual in need of a decreased level of METHI or METH2 activity in the body comprising, administering to such an individual a composition comprising a therapeutically effective amount ofMETH I or N1ETH2 antagonist. Preferred antagonists for use in the present invention are METH I or METH2-specific antibodies.

Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of METH I or METH2 in an individual can be treated by administering METH I or NEETH2, preferably in the secreted form, I 0 Thus, the invention also provides a method of treatment of an individual in need of an increased level ofMETH I or NIETH2 polypeptide comprising administering to such an individual a pharmaceutical composition comprising an amount of METH I or METH2 to increase the activity level of METH I or NMTH2 in such an individual.

1 5 For example, a patient with decreased levels of METHI or METH2 polypeptide receives a daily dose 0 100 ug/kg of the polypeptide for six consecutivedays. Preferably,thepolypeptideisinthesecretedform. Theexact details of the dosing scheme, based on administration and formulation, are provided in Example 26.

Example 28: Method of Treating Increased Levels qf METHI or METH2 The present invention also relates to a method for treating an individual in need of an increased level of METH I or METH2 activity in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of METH I or METH2 or an agonist thereof Antisense technology is used to inhibit production of METH I or METH2.

This technology is one example of a method of decreasing levels of METHI or METH2 polypeptide, preferably a secreted form, due to a variety of etiologies, such as cancer.

For example, a patient diagnosed with abnormally increased levels of NIETHI or METH2 is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 26.

Example 29: Method of Treatment Using Gene Therapy - Ex Vivo One method of gene therapy transplants fibroblasts, which are capable of expressing METHI or M]ETH2 polypeptides, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is I 0 placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask 1 5 and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge.

The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. el aL, DNA 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding METHI or METH2 can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set forth in Example 5. Preferably, the 5' primer contains an EcoRl site and the 3' primer includes a HindIll site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRl and HindIll fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector contains property inserted METHI or METH2.

The amphotropic pA317 or GP+am I 2 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing I 0 the METHI or METI-12 gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the METHI or METI-12 gene(the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a IO cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether METHI or METI-12 protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

Example 30: Method of Treatment Using Gene Therapy - In Vivo 1 1 Another aspect of the present invention is using in vivo gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) METH I or METH2 sequences into an animal to increase or decrease the expression of the NffiTHI or METH2 polypeptide. The METHI or METH2 polynucleotide may be operatively linked to a promoter or any other genetic elements necessary for the expression of the NMTH I or NMTH2 polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, W090/11092, W098/11779 U. S. Patent NO.

5 693 622, 5 70515 1, 5 5 808 5 9 -, Tabata H. et al. (I 997) Cardiovasc. Res. 35(3):470- 479, Chao, J et al. (1997) Pharmacol. Res. 35(6):517-522, Wolff J.A. (1997) Neuromuscul. Disord. 7(5):314-318, Schwartz, B. et al. (1996) Gene Ther.

3(5):405-411, Tsurumi Y. et al. (1996) Circulation 94(12):3281-3290 (incorporated herein by reference).

The METHI or METH2 polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The METH I or METH2 polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the METH I or METH2 polynucleotides may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (I 995)Ann. ATAcad. Sci. 772:126- 139 and Abdallah B. et al. (1995) Biol. Cell 85(l):1-7) which can be prepared by methods well known to those skilled in the art.

The METHI or METH2 polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genorne nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non- replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

I 0 The METH I or N4ETH2 polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues 1 5 comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells

are particularly competent in their ability to take up and express polynucleotides.

For the naked NMTHI or METH2 polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0. 05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the I 0 nose. In addition, naked NIEETHI or METH2 polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected METHI or NIETH2 polynucleotide in muscle in vivo is determined as follows. Suitable METHI or NIETH2 template DNA for production of mRNA coding for METHI or METH2 polypeptide is 1 5 prepared in accordance with a standard recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The METH I or METH2 template DNA is injected in 0. I ml of carrier in a I cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for METH I or METH2 protein expression. A time course for METH I or NMTH2 protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of METH I or NMTH2 DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using METHI or METH2 naked DNA, It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples.

I 0 Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of all publications (including patents, patent applications, journal articles, laboratory manuals, books, or other documents) cited herein are hereby incorporated by reference.

- Applicant's or agent's file International application iNo: TBA reference number: 1488.107PCO2 INDICATIONS RELATING TO A DEPOSITED MICROORGANISM (PCT Rule 13bis) A. The indications made below relate to the microoruanism referred to in the description on page 32. lines 16 B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet 0 Name of depository institution American Type Culture Collection Address of depository institution (including postal code and country) 10801 University Boulevard formerlv. at: 12301 Parklawn Drive Manassas, Virginia 20110-2209 Rockville, Maryland 20852 United States of America United States of America Date of deposit Accession Number 15 January 1998 209581 C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet El DNA plasmid HOUCQ 1 7 D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indicatzons are not for all designatedStates) E. SEPARATE FURNISHING OF INDICATIONS avatehlanA itnwqpphcahle@ The indications listed below will be submitted to the international Bureau later (specifv the general nature of the indications, e g, ",Accession Number of Deposit") For receivinp Off-ice use onl\ For International Bureau use on1% I'his sheet "as received with the international application 0 This sheet %%as received bY the International Bureau on Authorized officer Authorized officer Form PCTrR0/I 34 (Jul% 1992) 107expsoljp pci - Applicant's or agent's file International application No: TBA reference number: 1488.107PCO2 INDICATIONS RELATING TO A DEPOSITED

MICROORGANISM (PCT Rule 13bis) A. The indications made below relate to the microoroanism referred to in the description on page 32, lines 25 B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet Name of depositon- institution American Type Culture Collection Address of dcpository institution (including postal code and counu@v) 10801 University Boulevard formerly at: 12301 Parklawn Drive Manassas. Virginia 20110-2209 Rockville, Maryland 20852 United States of America United States of America Date of deposit Accession Number 15 January 1998 209582 C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet El DNA plasmid HCE4D69 D. DESIGNATEDSTATESFORWHICHINDICATIONSAREMA/6ralidestznaletiStates) E. SEPARATE FURNISHING OF INDICATIONS demehlank itnotapplicahle) The indications listed belo will be submitted to the international Bureau later apecift, the general nature of the indications. e.g..

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I . An isolated nucleic acid molecule comprising a polynucleotide selected from the group consisting of (a) apolynucleotide encoding a polypeptide comprising amino acids I to 950 in SEQ ID NO:2; (b) a polynucleotide encoding a polypeptide comprising amino acids 2 to 950 in SEQ ID NO:2; (c) apolynucleotide encoding apolypeptidecomprisingamino acids 29 to 950 in SEQ ID NO:2; (d) a polynucleotide encoding a polypeptide comprising amino acids 30 to 950 in SEQ ID NO:2@- (e) a polynucleotide comprising a nucleotide sequence encoding the METH I polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 2095 8 1; (O a polynucleotide comprising a nucleotide sequence encoding the mature METHI polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 2095 8 1; (g) a polynucleotide encoding a polypeptide comprising amino acids I to 890 in SEQ ID NOA; (h) apolynucleotide encoding apolypeptide comprising amino acids 2 to 890 in SEQ ID NOA7 (i) a polynucleotide encoding a polypeptide comprising amino acids 24 to 890 in SEQ ID NOA; 0) a polynucleotide encoding a polypeptide comprising amino acids 112 to 890 in SEQ ID NOA; (k) a polynucleotide comprising a nucleotide sequence encoding the METH2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209582; (1) a polynucleotide comprising a nucleotide sequence encoding the mature METH2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209582,- (in) a polynucleotide variant created by altering a polynucleotide of (a), (b), (c), (d), (e), (f), (g), (h), (i), 0), (k), or (1), wherein: (i) said altering includes a nucleotide insertion, deletion, or substitution, or any combination thereof, and (ii) the number of alterations is equal to or less than 5% of the total number of nucleotides present in the unaltered polynucleotide; I 0 (n) a polymicleotide encoding amino acids 23 5 to 45 9 in SEQ ID NO:2; (o) a polynucleotide encoding amino acids 460 to 544 in SEQ ID NO:2; (p) a polynucleotide encoding amino acids 545 to 598 in SEQ 1 5 ID NO:2; (q) a polynucleotide encoding amino acids 841 to 894 in SEQ ID NO: 2; (r) a polynucleotide encoding amino acids 895 to 934 in SEQ ID NO:2; (s) a polynucleotide encoding amino acids 53 6 to 613 in SEQ ID NO:2; (t) a polynucleotide encoding amino acids 549 to 563 in SEQ ID NO:2; (u) a polynucleotide encoding amino acids 214 to 43 9 in SEQ ID NOA; (v) a polynucleotide encoding amino acids 440 to 529 in SEQ ID NOA; (w) a polynucleotide encoding amino acids 530 to 583 in SEQ ID NOA; (x) a polynucleotide encoding amino acids 837 to 890 in SEQ ID NOA)- (y) a polynucleotide encoding amino acids 280 to 606 in SEQ ID NOA); (z) a polynucleotide encoding amino acids 529 to 548 in SEQ ID NOA; and (aa) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), 0), (k), (1), (in), (n), (o), (p), (q), (r), (s), (t), (u), (v), (w), (x), (y), or (z).

2 An isolated nucleic acid molecule comprising a polynucleotide which encodes the amino acid sequence of an epitope-bearing portion of the NffiTHI polypeptide of SEQ ID NO:2 or the METH2 polypeptide of SEQ ID NOA.

3 An isolated nucleic acid molecule, comprising a polynucleotide 1 5 selected from the group consisting of-. (a) 50 contiguous nucleotides of the coding region of SEQ ID NO: 1, provided that said nucleotide sequence is not any one of SEQ ID NOs: 14- 4 1, or any subfragment thereof, and (b) a nucleotide sequence complementary to the nucleotide sequence in (a).

4 An isolated nucleic acid molecule, comprising a polynucleotide selected from the group consisting of-. (a) 50 contiguous nucleotides of the coding region of SEQ ID NO:3, provided that said nucleotide sequence is not SEQ ID NOs: 19-22, 24, 42-

77 or any subfragment thereof, and (b) a nucleotide sequence complementary to the nucleotide sequence in (a). 5 A method for making a recombinant vector comprising inserting an isolated nucleic acid molecule of claim I into a vector in operable linkage to a promoter.

6 A recombinant vector produced by the method of claim 5.

7 A method of making a recombinant host cell comprising introducing the recombinant vector of claim 6 into a host cell.

8 A recombinant host cell produced by the method of claim 7,

9 A recombinant method for producing a NMTHI or METH2 polypeptide, comprising culturing the recombinant host cell of claim 8 under I 0 conditions such that said polypeptide is expressed and recovering said polypeptide.

10 An isolated polypeptide comprising an amino acid sequence selected from the group consisting of. (a) amino acids I to 950 in SEQ ID NO:2; (b) amino acids 2 to 950 in SEQ ID NO:2)- (c) amino acids 29 to 950 in SEQ ID NO:2; (d) amino acids 30 to 950 in SEQ ID NO:2; (d) the amino acid sequence ofthe WTH I polyp eptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209581; (e) the amino acid sequence ofthe mature METH I polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 20958 1; (f) amino acids I to 890 in SEQ ID NO:41 (g) amino acids 2 to 890 in SEQ ID NOA; (h) amino acids 24 to 890 in SEQ ID NOA) (1) amino acids 112 to 890 in SEQ ID NOA; 0) , an amino acid sequence of the METI-12 polypeptide having the amino acid sequence encoded by the NIETH2 cDNA clone contained in ATCC Deposit No. 209582-, (k) an amino acid sequence ofthe mature METH2 polypeptide having the amino acid sequence encoded by the METH2 cDNA clone contained in ATCC Deposit No. 209582; (1) the amino acid sequence of a polypeptide variant created by altering a polypeptide of (a), (b), (c), (d), (e), (f), (g), (h), (i), 0), or (k), wherein: (i) said altering includes an amino acid insertion, deletion, or substitution, or any combination thereof, and (ii) the number of alterations is equal to or less than 5% of the total number of amino acids present in the unaltered amino acid sequence; 1 5 (m) amino acids 235 to 459 in SEQ ID NO:2; (n) amino acids 460 to 544 in SEQ ID NO:2; (o) amino acids 545 to 598 in SEQ ID NO:2)- (p) amino acids 841 to 894 in SEQ ID NO:2; (q) amino acids 895 to 934 in SEQ ID NO:2; (r) amino acids 536 to 613 in SEQ ID NO:2; (s) amino acids 549 to 563 in SEQ ID NO:2; (t) amino acids 214 to 439 in SEQ ID NOA; (u) amino acids 440 to 529 in SEQ ID NOA; (v) amino acids 530 to 583 in SEQ ID NOA; (w) amino acids 837 to 890 in SEQ ID NOA@- (x) amino acids 280 to 606 in SEQ ID NOA; (y) amino acids 529 to 548 in SEQ ID NOA; (z) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), (e), (f), (g), (h), (i), 0), (k), (1) @ (in), (n), (o), (p), (q), (r), (s), (t), (u), (v), (w), (x), or (y).

11 The isolated polypeptide of claim 10, which is produced in a recombinant host cell.

12 The isolated polypeptide of claim I 1, wherein said recombinant host cell is mammalian.

13 An isolated nucleic acid molecule comprising a polynucleotide encoding a METHI or METH2 polypeptide wherein, except for one to fifty conservative amino acid substitutions, said polypeptide has a sequence selected from the group consisting of (a) amino acids from about I to about 950 in SEQ ID NO:2; (b) amino acids from about 2 to about 950 in SEQ ID NO:2@- (c) amino acids from about 29 to about 950 in SEQ ID NO:21 (d) amino acids from about 30 to about 950 in SEQ ID NO:2; (e) the amino acid sequence of the MEETHI polypeptide as encoded by the cl)NA clone contained in ATCC Deposit No. 2095 8 11 1 5 (f) the amino acid sequence ofthe mature METH I polypeptide as encoded by the cDNA clone contained in ATCC Deposit No. 2095 8 1; (g) amino acids from about I to about 890 in SEQ ID NOA) (h) amino acids from about 2 to about 890 in SEQ ID NOA; (i) amino acids from about 24 to 890 in SEQ ID NOA) 0) amino acids from about 112 to about 890 in SEQ ID NOA; (k) the amino acid sequence of the METH2 polypeptide as encoded by the cDNA clone contained in ATCC Deposit No. 209582- and (1) the amino acid sequence ofthe mature METH2 polypeptide as encoded by the cDNA clone contained in ATCC Deposit No. 209582.

14 An isolated polypeptide wherein, except for one to fifty conservative amino acid substitutions, said polypeptide has a sequence selected from the group consisting of (a) amino acids from about I to about 950 in SEQ ID NO:2; (b) amino acids from about 2 to about 950 in SEQ ID NO:2)- (c) amino acids from about 29 to about 950 in SEQ ID NO:2; (d) amino acids from about 3 0 to about 95 0 in SEQ ID NO: 2; (e) the amino acid sequence ofthe METH I polypeptide

having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209581; (f) the amino acid sequence ofthe mature METH I polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC I 0 Deposit No. 2095 8 1; (g) amino acids from about I to about 890 in SEQ ID NOA; (h) amino acids from about 2 to about 890 in SEQ ID NOA; (i) amino acids from about 24 to about 890 in SEQ ID NOA; 0) amino acids from about 112 to about 890 in SEQ ID NOA1 1 5 (k) the amino acid sequence ofthe METH2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209582; (1) the amino acid sequence ofthe mature METH2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209582; and (in) the amino acid sequence of an epitope-bearing portion of any one of the polypeptides of (a), (b), (c), (d), (e), (f), (g), (h), (i), 0), (k), or (1).

15 An isolated nucleic acid molecule comprising a polynucleotide at least 95% identical to a polynucleotide selected from the group consisting of (a) a polynucleotide encoding a polypeptide comprising amino acids I to 950 in SEQ ID NO:2@- (b) a polynucleotide encoding a polypeptide comprising amino acids 2 to 950 in SEQ ID NO:2)- (c) a polynucleotide encoding a polypeptide comprising amino acids 29 to 950 in SEQ ID NO:21 (d) a polynucleotide encoding a polypeptide comprising amino acids 30 to 950 in SEQ ID NO:2; (e) a polynucleotide comprising a nucleotide sequence encoding the MIETHI polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 2095 8 1; (f) a polynucleotide comprising a nucleotide sequence encoding the mature METHI polypeptide having the amino acid sequence I 0 encoded by the cDNA clone contained in ATCC Deposit No. 2095 8 1; (g) a polynucleotide encoding a polypeptide comprising amino acids I to 890 in SEQ ID NOA; (h) a polynucleotide encoding a polypeptide comprising amino acids 2 to 890 in SEQ ID NOA; (i) apolynucleotide encoding apolypeptidecomprising amino acids 24 to 890 in SEQ ID NOA; 0) apolynucleotide encoding apolypeptide comprising amino acids 112 to 890 in SEQ ID NOA; (k) a polynucleotide comprising a nucleotide sequence encoding the METI-12 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209582; (1) a polynucleotide comprising a nucleotide sequence encoding the mature METH2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209582; and (m) a nucleotide sequence complementary to any of the nucleotide sequences in (a), (b), (c), (d), (e), (f), (g), (h), (i), 0), (k), or (1), wherein said % identity is calculated using the FASTDB computer program, with the parameters: Matrix=Unitary, k-tuple=4, Mismatch Penalty=l, Joining Penalty=30, Randomization Group Length=O, Cutoff Score=l, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject nucleotide sequence, whichever is shorter.

16 An isolated polypeptide comprising a polypeptide having 95% identity to a polypeptide having an amino acid sequence selected from the group consisting of (a) amino acids from about I to about 950 in SEQ ID NO:2)- (b) amino acids from about 2 to about 950 in SEQ ID NO:2-) (c) amino acids from about 29 to about 950 in SEQ ID NO:2; (d) amino acids from about 30 to about 950 in SEQ ID NO:2; (e) the amino acid sequence ofthe METH I polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209581; (f) the amino acid sequence ofthe mature METH I polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC 5 Deposit No. 20958 1; (g) amino acids from about I to about 890 in SEQ ID NOA; (h) amino acids from about 2 to about 890 in SEQ ID NOA; (i) amino acids from about 24 to about 890 in SEQ ID NO-41 0) amino acids from about 112 to about 890 in SEQ ID NOA)- (k) the amino acid sequence ofthe METH2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209582- and (1) the amino acid sequence ofthe mature METH2 polypeptide having the amino acid sequence encoded by the cDNA clone contained in ATCC Deposit No. 209582,- wherein said % identity is calculated using the FASTDB computer program, with the parameters: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=l, Joining Penalty=20, Randomization Group Length=O, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

17 A method for inhibiting angiogenesis in an individual, comprising administering an effective amount of a polypeptide of claim IO to said individual.

18 A polypeptide comprising the amino acid sequence m-n of SEQ ID NO:2, wherein in is an integer of I to 950, and wherein n is an integer of 10 to 950.

19 A polypeptide comprising the amino acid sequence m-n of SEQ ID NOA wherein in is an integer of I to 890, and wherein n is an integer of 10 to I 0 890.

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